



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>C12N 15/31, C07K 14/29, A61K 39/02,</b> <b>C12Q 1/68</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 00/65063</b> <b>(43) International Publication Date:</b> 2 November 2000 (02.11.00)
<b>(21) International Application Number:</b> PCT/US00/10886 <b>(22) International Filing Date:</b> 21 April 2000 (21.04.00)  <b>(30) Priority Data:</b> 60/130,725                      22 April 1999 (22.04.99)                      US  <b>(71) Applicant:</b> UNIVERSITY OF FLORIDA [US/US]; 223 Grinter Hall, Gainesville, FL 32611 (US).  <b>(72) Inventors:</b> BARBET, Anthony, F.; 8803 S.W. 138th Street, Archer, FL 32618 (US). BOWIE, Michael, V.; 2847 S.W. 39th Avenue, Gainesville, FL 32608 (US). GANTA, Roman, Reddy; 1608 Little Kitten Avenue, Manhattan, KS 66503 (US). BURRIDGE, Michael, J.; 10021 S.W. 67th Drive, Gainesville, FL 32608 (US). MAHAN, Suman, M.; 71 Olwyn Avenue, Strathaven, Harare (ZW). MCGUIRE, Travis, C.; S.W. 920 Crestview, Pullman, WA 99163 (US). RURANGIRWA, Fred, R.; 2065 N.W. Turner Drive, Pullman, WA 99163 (US). MORELAND, Annie, L.; 4499 S.E. 82nd Street, Trenton, FL 32963 (US). SIMBI, Bigboy, H.; P.O. Box CY 551, Causeway, Harare (ZW). WHITMIRE, William, W.; 1079 Outlaw Trail, Hamilton, MT 59840 (US). ALLEMAN, Arthur, R.; 13337 S.W. 39th Avenue, Alachua, FL 32615 (US).		<b>(74) Agents:</b> EISENSCHENK, Frank, C. et al.; Saliwanchik, Lloyd & Saliwanchik, A Professional Association, Suite A-1, 2421 N.W. 41st Street, Gainesville, FL 32606-6669 (US).  <b>(81) Designated States:</b> AU, TZ, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> NUCLEIC ACID VACCINES AGAINST RICKETTSIAL DISEASES AND METHODS OF USE  <b>(57) Abstract</b> <p>Described are nucleic acid vaccines containing genes to protect animals or humans against rickettsial diseases. Also described are polypeptides and methods of using these polypeptides to detect antibodies to pathogens.</p>		

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DESCRIPTIONNUCLEIC ACID VACCINES AGAINST  
RICKETTSIAL DISEASES AND METHODS OF USE

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This invention was made with government support under USAID Grant No. LAG-1328-G-00-3030-00. The government has certain rights in this invention.

Technical Field

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This invention relates to nucleic acid vaccines for rickettsial diseases of animals, including humans.

Background of the Invention

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The rickettsias are a group of small bacteria commonly transmitted by arthropod vectors to man and animals, in which they may cause serious disease. The pathogens causing human rickettsial diseases include the agent of epidemic typhus, *Rickettsia prowazekii*, which has resulted in the deaths of millions of people during wartime and natural disasters. The causative agents of spotted fever, e.g., *Rickettsia rickettsii* and *Rickettsia conorii*, are also included within this group. Recently, new types of human rickettsial disease caused by members of the tribe *Ehrlichiae* have been described. *Ehrlichiae* infect leukocytes and endothelial cells of many different mammalian species, some of them causing serious human and veterinary diseases. Over 400 cases of human ehrlichiosis, including some fatalities, caused by *Ehrlichia chaffeensis* have now been reported. Clinical signs of human ehrlichiosis are similar to those of Rocky Mountain spotted fever, including fever, nausea, vomiting, headache, and rash.

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Heartwater is another infectious disease caused by a rickettsial pathogen, namely *Cowdria ruminantium*, and is transmitted by ticks of the genus *Amblyomma*. The disease occurs throughout most of Africa and has an estimated endemic area of about 5 million square miles. In endemic areas, heartwater is a latent infection in indigenous breeds of cattle that have been subjected to centuries of natural selection. The problems occur where the disease contacts susceptible or naive cattle and other ruminants. Heartwater has been confirmed to be on the island of Guadeloupe in the Caribbean and is spreading through the Caribbean Islands. The tick vectors responsible for spreading this disease are already present on the American mainland and threaten the livestock industry in North and South America.

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In acute cases of heartwater, animals exhibit a sudden rise in temperature, signs of anorexia, cessation of rumination, and nervous symptoms including staggering, muscle twitching, and convulsions. Death usually occurs during these convulsions. Peracute cases of the disease occur where the animal collapses and dies in convulsions having shown no preliminary symptoms. Mortality is high in susceptible animals. Angora sheep infected with the disease have a 90% mortality rate while susceptible cattle strains have up to a 60% mortality rate.

If detected early, tetracycline or chloramphenicol treatment are effective against rickettsial infections, but symptoms are similar to numerous other infections and there are no satisfactory diagnostic tests (Helmick, C., K. Bernard, L. D'Angelo [1984] *J. Infect. Dis.* 150:480).

Animals which have recovered from heartwater are resistant to further homologous, and in some cases heterologous, strain challenge. It has similarly been found that persons recovering from a rickettsial infection may develop a solid and lasting immunity. Individuals recovered from natural infections are often immune to multiple isolates and even species. For example, guinea pigs immunized with a recombinant *R. conorii* protein were partially protected even against *R. rickettsii* (Vishwanath, S., G. McDonald, N. Watkins [1990] *Infect. Immun.* 58:646). It is known that there is structural variation in rickettsial antigens between different geographical isolates. Thus, a functional recombinant vaccine against multiple isolates would need to contain multiple epitopes, e.g., protective T and B cell epitopes, shared between isolates. It is believed that serum antibodies do not play a significant role in the mechanism of immunity against rickettsia (Uilenberg, G. [1983] *Advances in Vet. Sci. and Comp. Med.* 27:427-480; Du Plessis, J.L. [1970] *Onderstepoort J. Vet. Res.* 37(3):147-150).

Vaccines based on inactivated or attenuated rickettsiae have been developed against certain rickettsial diseases, for example against *R. prowazekii* and *R. rickettsii*. However, these vaccines have major problems or disadvantages, including undesirable toxic reactions, difficulty in standardization, and expense (Woodward, T. [1981] "Rickettsial diseases: certain unsettled problems in their historical perspective." In *Rickettsia and Rickettsial Diseases*, W. Burgdorfer and R. Anacker, eds., Academic Press, New York, pp. 17-40).

A vaccine currently used in the control of heartwater is composed of live infected sheep blood. This vaccine also has several disadvantages. First, expertise is required for the intravenous inoculation techniques required to administer this vaccine. Second, vaccinated animals may experience shock and so require daily monitoring for a period after vaccination. There is a possibility of death due to shock throughout this monitoring period, and the drugs

needed to treat any shock induced by vaccination are costly. Third, blood-borne parasites may be present in the blood vaccine and be transmitted to the vaccinees. Finally, the blood vaccine requires a cold chain to preserve the vaccine.

Clearly, a safer, more effective vaccine that is easily administered would be particularly advantageous. For these reasons, and with the advent of new methods in biotechnology, investigators have concentrated recently on the development of new types of vaccines, including recombinant vaccines. However, recombinant vaccine antigens must be carefully selected and presented to the immune system such that shared epitopes are recognized. These factors have contributed to the search for effective vaccines.

A protective vaccine against rickettsiae that elicits a complete immune response can be advantageous. A few antigens which potentially can be useful as vaccines have now been identified and sequenced for various pathogenic rickettsia. The genes encoding the antigens and that can be employed to recombinantly produce those antigen have also been identified and sequenced. Certain protective antigens identified for *R. rickettsii*, *R. conorii*, and *R. prowazekii* (e.g., rOmpA and rOmpB) are large (>100 kDa), dependent on retention of native conformation for protective efficacy, but are often degraded when produced in recombinant systems. This presents technical and quality-control problems if purified recombinant proteins are to be included in a vaccine. The mode of presentation of a recombinant antigen to the immune system can also be an important factor in the immune response.

Nucleic acid vaccination has been shown to induce protective immune responses in non-viral systems and in diverse animal species (Special Conference Issue, WHO meeting on nucleic acid vaccines [1994] *Vaccine* 12:1491). Nucleic acid vaccination has induced cytotoxic lymphocyte (CTL), T-helper 1, and antibody responses, and has been shown to be protective against disease (Ulmer, J., J. Donnelly, S. Parker *et al.* [1993] *Science* 259:1745). For example, direct intramuscular injection of mice with DNA encoding the influenza nucleoprotein caused the production of high titer antibodies, nucleoprotein-specific CTLs, and protection against viral challenge. Immunization of mice with plasmid DNA encoding the *Plasmodium yoelii* circumsporozoite protein induced high antibody titers against malaria sporozoites and CTLs, and protection against challenge infection (Sedegah, M., R. Hedstrom, P. Hobart, S. Hoffman [1994] *Proc. Natl. Acad. Sci. USA* 91:9866). Cattle immunized with plasmids encoding bovine herpesvirus 1 (BHV-1) glycoprotein IV developed neutralizing antibody and were partially protected (Cox, G., T. Zamb, L. Babiuk [1993] *J. Virol.* 67:5664). However, it has been a question in the field of immunization whether the recently discovered technology of nucleic acid vaccines can provide improved protection against an antigenic drift variant. Moreover, it has

not heretofore been recognized or suggested that nucleic acid vaccines may be successful to protect against rickettsial disease or that a major surface protein conserved in rickettsia was protective against disease.

#### Brief Summary of the Invention

Disclosed and claimed here are novel vaccines for conferring immunity to rickettsia infection, including *Cowdria ruminantium* causing heartwater. Also disclosed are novel nucleic acid compositions and methods of using those compositions, including to confer immunity in a susceptible host. Also disclosed are novel materials and methods for diagnosing infections by *Ehrlichia* in humans or animals.

One aspect of the subject invention concerns a nucleic acid, *e.g.*, DNA or mRNA, vaccine containing the major antigenic protein 1 gene (MAP1) or the major antigenic protein 2 gene (MAP2) of rickettsial pathogens. In one embodiment, the nucleic acid vaccines can be driven by the human cytomegalovirus (HCMV) enhancer-promoter. In studies immunizing mice by intramuscular injection of a DNA vaccine composition according to the subject invention, immunized mice seroconverted and reacted with MAP1 in antigen blots. Splenocytes from immunized mice, but not from control mice immunized with vector only, proliferated in response to recombinant MAP1 and rickettsial antigens in *in vitro* lymphocyte proliferation tests. In experiments testing different DNA vaccine dose regimens, increased survival rates as compared to controls were observed on challenge with rickettsia. Accordingly, the subject invention concerns the discovery that DNA vaccines can induce protective immunity against rickettsial disease or death resulting therefrom.

The subject invention further concerns the genes designated *Cowdria ruminantium map 2*, *Cowdria ruminantium 1hworf3*, *Cowdria ruminantium 4hworf1*, *Cowdria ruminantium 18hworf1*, and *Cowdria ruminantium 3gdorf3* and the use of these genes in diagnostic and therapeutic applications. The subject invention further concerns the proteins encoded by the exemplified genes, antibodies to these proteins, and the use of such antibodies and proteins in diagnostic and therapeutic applications.

In one embodiment of the subject invention, the polynucleotide vaccines are administered in conjunction with an antigen. In a preferred embodiment, the antigen is the polypeptide which is encoded by the polynucleotide administered as the polynucleotide vaccine. As a particularly preferred embodiment, the antigen is administered as a booster subsequent to the initial administration of the polynucleotide vaccine.

### Brief Description of the Drawings

**Figures 1A-1C** show a comparison of the amino acid sequences from alignment of the three rickettsial proteins, namely, *Cowdria ruminantium* (C.r.), *Ehrlichia chaffeensis* (E.c.), and *Anaplasma marginale* (A.m.).

**Figures 2A-2C** shows the DNA sequence of the 28 kDa gene locus cloned from *E. chaffeensis* (Fig. 2A-2B) and *E. canis* (Fig. 2C). One letter amino acid codes for the deduced protein sequences are presented below the nucleotide sequence. The proposed sigma-70-like promoter sequences (38) are presented in bold and underlined text as -10 and -35 (consensus -35 and -10 sequences are TTGACA and TATAAT, respectively). Similarly, consensus ribosomal binding sites and transcription terminator sequences (bold letter sequence) are identified. G-rich regions identified in the *E. chaffeensis* sequence are underlined. The conserved sequences from within the coding regions selected for RT-PCR assay are identified with italics and underlined text.

**Figure 3A** shows the complete sequence of the MAP2 homolog of *Ehrlichia canis*. The arrow (→) represents the predicted start of the mature protein. The asterisk (\*) represents the stop codon. Underlined nucleotides 5' to the open reading frame with -35 and -10 below represent predicted promoter sequences. Double underlined nucleotides represent the predicted ribosomal binding site. Underlined nucleotides 3' to the open reading frame represent possible transcription termination sequences.

**Figure 3B** shows the complete sequence of the MAP2 homolog of *Ehrlichia chaffeensis*. The arrow (→) represents the predicted start of the mature protein. The asterisk (\*) represents the stop codon. Underlined nucleotides 5' to the open reading frame with -35 and -10 below represent predicted promoter sequences. Double underlined nucleotides represent the predicted ribosomal binding site. Underlined nucleotides 3' to the open reading frame represent possible transcription termination sequences.

### Brief Description of the Sequences

**SEQ ID NO. 1** is the coding sequence of the MAP1 gene from *Cowdria ruminantium* (Highway isolate).

**SEQ ID NO. 2** is the polypeptide encoded by the polynucleotide of SEQ ID NO. 1.

**SEQ ID NO. 3** is the coding sequence of the MAP1 gene from *Ehrlichia chaffeensis*.

**SEQ ID NO. 4** is the polypeptide encoded by the polynucleotide of SEQ ID NO. 3.

**SEQ ID NO. 5** is the *Anaplasma marginale* MSP4 gene coding sequence.

**SEQ ID NO. 6** is the polypeptide encoded by the polynucleotide of SEQ ID NO. 5.

SEQ ID NO. 7 is a partial coding sequence of the VSA1 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

SEQ ID NO. 8 is the coding sequence of the VSA2 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

5 SEQ ID NO. 9 is the coding sequence of the VSA3 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

SEQ ID NO. 10 is the coding sequence of the VSA4 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

10 SEQ ID NO. 11 is a partial coding sequence of the VSA5 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

SEQ ID NO. 12 is the coding sequence of the VSA1 gene from *Ehrlichia canis*, also shown in Figure 2C.

SEQ ID NO. 13 is a partial coding sequence of the VSA2 gene from *Ehrlichia canis*, also shown in Figure 2C.

15 SEQ ID NO. 14 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 7, also shown in Figures 2A-2B.

SEQ ID NO. 15 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 8, also shown in Figures 2A-2B.

20 SEQ ID NO. 16 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 9, also shown in Figures 2A-2B.

SEQ ID NO. 17 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 10, also shown in Figures 2A-2B.

SEQ ID NO. 18 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 11, also shown in Figures 2A-2B.

25 SEQ ID NO. 19 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 12, also shown in Figure 2C.

SEQ ID NO. 20 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 13, also shown in Figure 2C.

30 SEQ ID NO. 21 is the coding sequence of the MAP2 gene from *Ehrlichia canis*, also shown in Figure 3A.

SEQ ID NO. 22 is the coding sequence of the MAP2 gene from *Ehrlichia chaffeensis*, also shown in Figure 3B.

SEQ ID NO. 23 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 21, also shown in Figure 3A.

SEQ ID NO. 24 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 22, also shown in Figure 3B.

SEQ ID NO. 25 is the coding sequence of the *map2* gene from *Cowdria ruminantium*.

SEQ ID NO. 26 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 25.

5 SEQ ID NO. 27 is the coding sequence of the *ihworf3* gene from *Cowdria ruminantium*.

SEQ ID NO. 28 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 27.

SEQ ID NO. 29 is the coding sequence of the *4hworf1* gene from *Cowdria ruminantium*.

SEQ ID NO. 30 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 29.

10 SEQ ID NO. 31 is the coding sequence of the *18hworf1* gene from *Cowdria ruminantium*.

SEQ ID NO. 32 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 31.

SEQ ID NO. 33 is the coding sequence of the *3gdorf3* gene from *Cowdria ruminantium*.

SEQ ID NO. 34 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 33.

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#### Detailed Disclosure of the Invention

In one embodiment, the subject invention concerns a novel strategy, termed nucleic acid vaccination, for eliciting an immune response protective against rickettsial disease. The subject invention also concerns novel compositions that can be employed according to this novel strategy for eliciting a protective immune response.

20 According to the subject invention, recombinant DNA or mRNA encoding an antigen of interest is inoculated directly into the human or animal host where an immune response is induced. Prokaryotic signal sequences may be deleted from the nucleic acid encoding an antigen of interest. Advantageously, problems of protein purification, as can be encountered with antigen delivery using live vectors, can be virtually eliminated by employing the compositions or methods according to the subject invention. Unlike live vector delivery, the subject invention can provide a further advantage in that the DNA or RNA does not replicate in the host, but remains episomal. See, for example, Wolff, J.A., J.J. Ludike, G. Acsadi, P. Williams, A. Jani (1992) *Hum. Mol. Genet.* 1:363. A complete immune response can be obtained as recombinant antigen is synthesized intracellularly and presented to the host immune system in the context of autologous class I and class II MHC molecules.

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In one embodiment, the subject invention concerns nucleic acids and compositions comprising those nucleic acids that can be effective in protecting an animal from disease or death caused by rickettsia. For example, a nucleic acid vaccine of the subject invention has been

shown to be protective against *Cowdria ruminantium*, the causative agent of heartwater in domestic ruminants. Accordingly, nucleotide sequences of rickettsial genes, as described herein, can be used as nucleic acid vaccines against human and animal rickettsial diseases.

In one embodiment of the subject invention, the polynucleotide vaccines are administered in conjunction with an antigen. In a preferred embodiment, the antigen is the polypeptide which is encoded by the polynucleotide administered as the polynucleotide vaccine. As a particularly preferred embodiment, the antigen is administered as a booster subsequent to the initial administration of the polynucleotide vaccine. In another embodiment of the invention, the polynucleotide vaccine is administered in the form of a "cocktail" which contains at least two of the nucleic acid vaccines of the subject invention. The "cocktail" may be administered in conjunction with an antigen or an antigen booster as described above.

The MAP1 gene, which can be used to obtain this protection, is also present in other rickettsiae including *Anaplasma marginale*, *Ehrlichia canis*, and in a causative agent of human ehrlichiosis, *Ehrlichia chaffeensis* (van Vliet, A., F. Jongejan, M. van Kleef, B. van der Zeijst [1994] *Infect. Immun.* 62:1451). The MAP1 gene or a MAP1-like gene can also be found in certain *Rickettsia* spp. MAP1-like genes from *Ehrlichia chaffeensis* and *Ehrlichia canis* have now been cloned and sequenced. These MAP-1 homologs are also referred to herein as Variable Surface Antigen (VSA) genes.

The present invention also concerns polynucleotides encoding MAP2 or MAP2 homologs from *Ehrlichia canis* and *Ehrlichia chaffeensis*. MAP2 polynucleotide sequences of the invention can be used as vaccine compositions and in diagnostic assays. The polynucleotides can also be used to produce the MAP2 polypeptides encoded thereby.

The subject invention further concerns the genes designated *Cowdria ruminantium map 2*, *Cowdria ruminantium 1hworf3*, *Cowdria ruminantium 4hworf1*, *Cowdria ruminantium 18hworf1*, and *Cowdria ruminantium 3gdorf3* and the use of these genes in diagnostic and therapeutic applications. The subject invention further concerns the proteins encoded by the exemplified genes, antibodies to these proteins, and the use of such antibodies and proteins in diagnostic and therapeutic applications.

Compositions comprising the subject polynucleotides can include appropriate nucleic acid vaccine vectors (plasmids), which are commercially available (e.g., Vical, San Diego, CA). In addition, the compositions can include a pharmaceutically acceptable carrier, e.g., saline. The pharmaceutically acceptable carriers are well known in the art and also are commercially available. For example, such acceptable carriers are described in E.W. Martin's *Remington's Pharmaceutical Science*, Mack Publishing Company, Easton, PA.

The subject invention also concerns polypeptides encoded by the subject polynucleotides. Specifically exemplified are the polypeptides encoded by the MAP-1 and VSA genes of *C. rumimontium*, *E. chaffeensis*, *E. canis* and the MP4 gene of *Anaplasma marginale*. Polypeptides encoded by *E. chaffeensis* and *E. canis* MAP2 genes are also exemplified herein.

Also encompassed within the scope of the present invention are fragments and variants of the exemplified polynucleotides and polypeptides. Fragments would include, for example, portions of the exemplified sequences wherein procaryotic signal sequences have been removed. Examples of the removal of such sequences are given in Example 3. Variants include polynucleotides and/or polypeptides having base or amino acid additions, deletions and substitutions in the sequence of the subject molecule so long as those variants have substantially the same activity or serologic reactivity as the native molecules. Also included are allelic variants of the subject polynucleotides. The polypeptides of the present invention can be used to raise antibodies that are reactive with the polypeptides disclosed herein. The polypeptides and polynucleotides can also be used as molecular weight markers.

Another aspect of the subject invention concerns antibodies reactive with MAP-1 and MAP2 polypeptides disclosed herein. Antibodies can be monoclonal or polyclonal and can be produced using standard techniques known in the art. Antibodies of the invention can be used in diagnostic and therapeutic applications.

In a specific embodiment, the subject invention concerns a DNA vaccine (e.g., VCL1010/MAP1) containing the major antigenic protein 1 gene (MAP1) driven by the human cytomegalovirus (HCMV) enhancer-promoter. In a specific example, this vaccine was injected intramuscularly into 8-10 week-old female DBA/2 mice after treating them with 50  $\mu$ l/muscle of 0.5% bupivacaine 3 days previously. Up to 75% of the VCL1010/MAP1-immunized mice seroconverted and reacted with MAP1 in antigen blots. Splenocytes from immunized mice, but not from control mice immunized with VCL1010 DNA (plasmid vector, Vical, San Diego) proliferated in response to recombinant MAP1 and *C. ruminantium* antigens in *in vitro* lymphocyte proliferation tests. These proliferating cells from mice immunized with VCL1010/MAP1 DNA secreted IFN-gamma and IL-2 at concentrations ranging from 610 pg/ml and 152 pg/ml to 1290 pg/ml and 310 pg/ml, respectively. In experiments testing different VCL1010/MAP1 DNA vaccine dose regimens (25-100  $\mu$ g/dose, 2 or 4 immunizations), survival rates of 23% to 88% (35/92 survivors/total in all VCL1010/MAP1 immunized groups) were observed on challenge with 30LD50 of *C. ruminantium*. Survival rates of 0% to 3% (1/144 survivors/total in all control groups) were recorded for control mice immunized similarly with VCL1010 DNA or saline. Accordingly, in a specific embodiment, the subject invention

concerns the discovery that the gene encoding the MAP1 protein induces protective immunity as a DNA vaccine against rickettsial disease.

The nucleic acid sequences described herein have other uses as well. For example, the nucleic acids of the subject invention can be useful as probes to identify complementary sequences within other nucleic acid molecules or genomes. Such use of probes can be applied to identify or distinguish infectious strains of organisms in diagnostic procedures or in rickettsial research where identification of particular organisms or strains is needed. As is well known in the art, probes can be made by labeling the nucleic acid sequences of interest according to accepted nucleic acid labeling procedures and techniques. A person of ordinary skill in the art would recognize that variations or fragments of the disclosed sequences which can specifically and selectively hybridize to the DNA of rickettsia can also function as a probe. It is within the ordinary skill of persons in the art, and does not require undue experimentation in view of the description provided herein, to determine whether a segment of the claimed DNA sequences is a fragment or variant which has characteristics of the full sequence, *e.g.*, whether it specifically and selectively hybridizes or can confer protection against rickettsial infection in accordance with the subject invention. In addition, with the benefit of the subject disclosure describing the specific sequences, it is within the ordinary skill of those persons in the art to label hybridizing sequences to produce a probe.

Various degrees of stringency of hybridization can be employed. The more severe the conditions, the greater the complementarity that is required for duplex formation. Severity of conditions can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*, Stockton Press, New York, NY., pp. 169-170.

Examples of various stringency conditions are provided herein. Hybridization of immobilized DNA on Southern blots with <sup>32</sup>P-labeled gene-specific probes can be performed by standard methods (Maniatis *et al.* (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York.). In general, hybridization and subsequent washes can be carried out under moderate to high stringency conditions that allow for detection of target sequences with homology to the exemplified polynucleotide sequence. For double-stranded DNA gene probes, hybridization can be carried out overnight at 20-25° C below the melting temperature (*T<sub>m</sub>*) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula (Beltz *et al.*

*et al.* [1983] *Methods of Enzymology*, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285).

$T_m = 81.5^{\circ}\text{C} - 16.6 \log[\text{Na}^+] + 0.41(\%G+C) - 0.61(\%\text{formamide}) - 600/\text{length of duplex in base pairs}$ .

5 Washes are typically carried out as follows:

- (1) twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash);
- (2) once at  $T_m - 20^{\circ}\text{C}$  for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

10 For oligonucleotide probes, hybridization can be carried out overnight at  $10-20^{\circ}\text{C}$  below the melting temperature ( $T_m$ ) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA.  $T_m$  for oligonucleotide probes can be determined by the following formula:

$T_m (^{\circ}\text{C}) = 2(\text{number T/A base pairs}) + 4(\text{number G/C base pairs})$  (Suggs *et al.* [1981] *ICN-UCLA Symp. Dev. Biol. Using Purified Genes*, D.D. Brown [ed.], Academic Press, New York, 23:683-693).

Washes can be carried out as follows:

- (1) twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash);
- 20 (2) once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment  $>70$  or so bases in length, the following conditions can be used:

- |    |           |                                       |
|----|-----------|---------------------------------------|
|    | Low:      | 1 or 2X SSPE, room temperature        |
| 25 | Low:      | 1 or 2X SSPE, $42^{\circ}\text{C}$    |
|    | Moderate: | 0.2X or 1X SSPE, $65^{\circ}\text{C}$ |
|    | High:     | 0.1X SSPE, $65^{\circ}\text{C}$ .     |

Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probe sequences of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions and deletions can be produced in a given

30

polynucleotide sequence in many ways, and these methods are known to an ordinarily skilled artisan. Other methods may become known in the future.

It is also well known in the art that restriction enzymes can be used to obtain functional fragments of the subject DNA sequences. For example, *Bal31* exonuclease can be conveniently used for time-controlled limited digestion of DNA (commonly referred to as "erase-a-base" procedures). See, for example, Maniatis *et al.* (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York; Wei *et al.* (1983) *J. Biol. Chem.* 258:13006-13512.

In addition, the nucleic acid sequences of the subject invention can be used as molecular weight markers in nucleic acid analysis procedures.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

#### Example 1

A nucleic acid vaccine construct was tested in animals for its ability to protect against death caused by infection with the rickettsia *Cowdria ruminantium*. The vaccine construct tested was the MAP1 gene of *C. ruminantium* inserted into plasmid VCL1010 (Vical, San Diego) under control of the human cytomegalovirus promoter-enhancer and intron A. In this study, seven groups containing 10 mice each were injected twice at 2-week intervals with either 100, 75, 50, or 25 µg VCL1010/MAP1 DNA (V/M in Table 1 below), or 100, 50 µg VCL1010 DNA (V in Table 1) or saline (Sal.), respectively. Two weeks after the last injections, 8 mice/group were challenged with 30LD50 of *C. ruminantium* and clinical symptoms and survival monitored. The remaining 2 mice/group were not challenged and were used for lymphocyte proliferation tests and cytokine measurements. The results of the study are summarized in Table 1, below:

Table 1

	100 µg V/M	75 µg V/M	50 µg V/M	25 µg V/M	100 µg V	50 µg V	Sal.
Survived	5	7	5	3	0	0	0
Died	3	1	3	5	8	8	8

The VCL1010/MAP1 nucleic acid vaccine increased survival on challenge in all groups, with a total of 20/30 mice surviving compared to 0/24 in the control groups.

This study was repeated with another 6 groups, each containing 33 mice (a total of 198 mice). Three groups received 75 µg VCL1010/MAP1 DNA or VCL1010 DNA or saline (4 injections in all cases). Two weeks after the last injection, 30 mice/group were challenged with 30LD50 of *C. ruminantium* and 3 mice/group were sacrificed for lymphocyte proliferation tests and cytokine measurements. The results of this study are summarized in Table 2, below:

Table 2						
	V/M 2 inj.	V 2 inj.	Sal. 2 inj.	V/M 4 inj.	V 4 inj.	Sal. 4 inj.
Survived	7	0	0	8	0	1
Died*	23	30	30	22	30	29

\*In mice that died in both V/M groups, there was an increase in mean survival time of approximately 4 days compared to the controls ( $p < 0.05$ ).

Again, as summarized in Table 2, the VCL1010/MAP1 DNA vaccine increased the numbers of mice surviving in both immunized groups, although there was no apparent benefit of 2 additional injections. In these two experiments, there were a cumulative total of 35/92 (38%) surviving mice in groups receiving the VCL1010/MAP1 DNA vaccine compared to 1/144 (0.7%) surviving mice in the control groups. In both immunization and challenge trials described above, splenocytes from VCL1010/MAP1 immunized mice, but not from control mice, specifically proliferated to recombinant MAP1 protein and to *C. ruminantium* in lymphocyte proliferation tests. These proliferating splenocytes secreted IL-2 and gamma-interferon at concentrations up to 310 and 1290 pg/ml respectively. These data show that protection against rickettsial infections can be achieved with a DNA vaccine. In addition, these experiments show MAP1-related proteins as vaccine targets.

#### Example 2 – Cloning and sequence analysis of MAP1 homologue genes of *E. chaffeensis* and *E. canis*

Genes homologous to the major surface protein of *C. ruminantium* MAP1 were cloned from *E. chaffeensis* and *E. canis* by using PCR cloning strategies. The cloned segments represent a 4.6 kb genomic locus of *E. chaffeensis* and a 1.6 kb locus of *E. canis*. DNA sequence generated from these clones was assembled and is presented along with the deduced amino acid

sequence in Figures 2A-2B (SEQ ID NOs. 7-11 and 14-18) and Figure 2C (SEQ ID NOs. 12-13 and 19-20). Significant features of the DNA include five very similar but nonidentical open reading frames (ORFs) for *E. chaffeensis* and two very similar, nonidentical ORFs for the *E. canis* cloned locus. The ORFs for both *Ehrlichia* spp. are separated by noncoding sequences ranging from 264 to 310 base pairs. The noncoding sequences have a higher A+T content (71.6% for *E. chaffeensis* and 76.1% for *E. canis*) than do the coding sequences (63.5% for *E. chaffeensis* and 68.0% for *E. canis*). A G-rich region -200 bases upstream from the initiation codon, sigma-70-like promoter sequences, putative ribosome binding sites (RBS), termination codons, and palindromic sequences near the termination codons are found in each of the *E. chaffeensis* noncoding sequences. The *E. canis* noncoding sequence has the same feature except for the G-rich region (Figure 2C: SEQ ID NOs. 12-13 and 19-20).

Sequence comparisons of the ORFs at the nucleotide and translated amino acid levels revealed a high degree of similarity between them. The similarity spanned the entire coding sequences, except in three regions where notable sequence variations were observed including some deletions/insertions (Variable Regions I, II and III). Despite the similarities, no two ORFs are identical. The cloned ORF 2, 3 and 4 of *E. chaffeensis* have complete coding sequences. The ORF1 is a partial gene having only 143 amino acids at the C-terminus whereas the ORF5 is nearly complete but lacks 5-7 amino acids and a termination codon. The cloned ORF2 of *E. canis* also is a partial gene lacking a part of the C-terminal sequence. The overall similarity between different ORFs at the amino acid level is 56.0% to 85.4% for *E. chaffeensis*, whereas for *E. canis* it is 53.3%. The similarity of *E. chaffeensis* ORFs to the MAP1 coding sequences reported for *C. ruminantium* isolates ranged from 55.5% to 66.7%, while for *E. canis* to *C. ruminantium* it is 48.5% to 54.2%. Due to their high degree of similarity to MAP1 surface antigen genes of *C. ruminantium* and since they are nonidentical to each other, the *E. chaffeensis* and *E. canis* ORFs are referred to herein as putative Variable Surface Antigen (VSA) genes. The apparent molecular masses of the predicted mature proteins of *E. chaffeensis* were 28.75 kDa for VSA2, 27.78 for VSA3, and 27.95 for VSA4, while *E. canis* VSA1 was slightly higher at 29.03 kDa. The first 25 amino acids in each VSA coding sequence were eliminated when calculating the protein size since they markedly resembled the signal sequence of *C. ruminantium* MAP1 and presumably would be absent from the mature protein.

The amino acid sequence derived from the cloned *E. chaffeensis* MAP1-like gene, and alignment with the corresponding genes of *C. ruminantium* and *A. marginale* is shown in Figure 1.

Example 3

A further aspect of the subject invention are five additional genes which give protection when formatted as DNA vaccines. These genes are *Cowdria ruminantium* map 2, *Cowdria ruminantium* 1hworf3, *Cowdria ruminantium* 4hworf1, *Cowdria ruminantium* 18hworf1, and  
5 *Cowdria ruminantium* 3gdorf3. The DNA and translated amino acid sequences of these five genes are shown in SEQ ID NOS. 25-34.

There is published information showing that gene homologs of all five genes are present in other bacteria. For example, a homolog of map2 is present in *Anaplasma marginale*, a homolog of 1hworf3 is present in *Brucella abortus*, homologs of 4hworf1 are present in  
10 *Pseudomonas aeruginosa* and *Coxiella burnetii*, and homologs of 18hworf1 are present in *Coxiella burnetii* and *Rickettsia prowazekii*. This can be revealed by a search of DNA and protein databases with standard search algorithms such as "Blast". Based on the protective ability of these genes against *Cowdria ruminantium* and their presence in other bacterial pathogens, the subject invention further concerns the use of these genes, their gene products, and  
15 the genes and gene products of the homologs as vaccines against bacteria. This includes their use as DNA or nucleic acid vaccines or when formulated in vaccines employing other methods of delivery, e.g., recombinant proteins or synthetic peptides in adjuvants, recombinant live vector delivery systems such as vaccinia (or other live viruses) or *Salmonella* (or other live bacteria). These methods of delivery are standard to those familiar with the field. This also includes  
20 vaccines against heartwater disease, vaccines against rickettsial diseases in general and vaccines against other bacteria containing homologs of these genes.

Table 3 shows the protective ability of the 5 genes against death from *Cowdria ruminantium* challenge in mice. Genes were inserted into VR1012 according to the manufacturers instructions (Vical, San Diego) and challenge studies were conducted as described  
25 in Example 1. N-terminal sequences which putatively encoded prokaryotic signal peptides were deleted because of the potential for their effects on expression and immune responses in eukaryotic expression systems or challenged animals. The inserts were as follows: map2, SEQ ID NO. 25, beginning at base 46; 18hworf1, SEQ ID NO. 31, beginning at base 67; 3gdorf3, SEQ ID NO. 33, beginning at base 79; 1hworf3, SEQ ID NO. 27, beginning at base 76; and  
30 4hworf1, SEQ ID NO. 29, beginning at base 58.

Table 3

DNA Construct	MWT Size	Survival Rate				
		Vaccinated		Control		P value
TMMAP 2	21 kd	9/28*	32%	0/29	0%	0.004
MB18HWORF1	28 kd	10/30*	33%	1/27	4%	0.021
AM3GDORF3	16 kd	7/26	27%	1/27	4%	0.060
TM1HWORF3	36 kd	8/29	28%	2/30	7%	0.093
TM4HWORF1	19 kd	10/30*	33%	2/30	7%	0.054

Control - VR1012 DNA vector plasmid only

\*Statistically significant difference (Fisher's Exact test)

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

Claims

1           1. A composition comprising a polynucleotide which encodes a polypeptide having the  
2 characteristic of eliciting an immune response protective against disease or death caused by a  
3 rickettsial pathogen.

1           2. The composition, according to claim 1, wherein said rickettsial pathogen is selected  
2 from the group consisting of *Rickettsia* spp., *Ehrlichia* spp., *Anaplasma* spp., and *Cowdria* spp.

1           3. The composition, according to claim 1, wherein said polypeptide has an amino acid  
2 sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6,  
3 SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NOS. 16-20, SEQ ID NO. 23, SEQ ID NO. 24, SEQ  
4 ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, homologs  
5 thereof, and immunogenic fragments thereof.

1           4. The composition, according to claim 1, wherein said polynucleotide has a nucleic  
2 acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO.  
3 5, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NOS. 9-13, SEQ ID NO. 21, SEQ ID NO. 22, . SEQ  
4 ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, homologs  
5 thereof, and fragments thereof which encode immunogenic polypeptides.

1           5. The composition, according to claim 4, wherein said polynucleotide has a nucleic  
2 acid sequence of SEQ ID NO. 3, or a fragment thereof.

1           6. The composition, according to claim 1, wherein said polynucleotide further  
2 comprises a nucleic acid vaccine vector.

1           7. The composition, according to claim 1, further comprising a pharmaceutically  
2 acceptable carrier.

1           8. A polynucleotide encoding a polypeptide having an amino acid sequence selected  
2 from the group consisting of SEQ ID NO. 4, SEQ ID NOS. 14-20, SEQ ID NOS. 23-24, SEQ  
3 ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, and fragments  
4 thereof.

1           9. The polynucleotide, according to claim 8, said polynucleotide having a nucleic acid  
2           sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NOS. 7-13, SEQ ID  
3           NOS. 21-22, SEQ ID NOS. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, and SEQ ID  
4           NO. 33.

1           10. A method for protecting a susceptible host against disease or death caused by a  
2           rickettsial pathogen, said method comprising administering an effective amount of a  
3           polynucleotide encoding polypeptide having the characteristic of eliciting an immune response  
4           protective against said rickettsial pathogen.

1           11. The method, according to claim 10, wherein said rickettsial pathogen is selected  
2           from the group consisting of *Rickettsia* spp., *Ehrlichia* spp., *Anaplasma* spp., and *Cowdria* spp.

1           12. The method, according to claim 10, wherein said polypeptide has an amino acid  
2           sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6,  
3           SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NOS. 16-20, SEQ ID NO. 23, SEQ ID NO. 24, SEQ  
4           ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, or homologs  
5           thereof and immunogenic fragments thereof.

1           13. The method, according to claim 10, wherein said polynucleotide has a nucleic acid  
2           sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5,  
3           SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NOS. 9-13, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID  
4           NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, and SEQ ID NO. 33.

1           14. The method, according to claim 13, wherein said polynucleotide has the nucleic acid  
2           sequence of SEQ ID NO. 1.

1           15. The method, according to claim 13, wherein said polynucleotide has the nucleic acid  
2           sequence of SEQ ID NO. 3.

1           16. The method, according to claim 13, wherein said polynucleotide has the nucleic acid  
2           sequence of SEQ ID NO. 5.

1           17. The method, according to claim 10, wherein said nucleic acid further comprises an  
2 appropriate nucleic acid vector.

1           18. The method, according to claim 10, wherein said composition further comprises a  
2 pharmaceutically acceptable carrier.

1           19. The method, according to claim 10, which further comprises administration to said  
2 host of said polypeptide encoded by said polypeptide.

1           20. A method for detecting, in a human or animal, antibodies associated with infection  
2 by *Ehrlichia*, wherein said method comprises contacting a biological fluid from said human or  
3 animal with a polypeptide selected from the group consisting of SEQ ID NO. 4, SEQ ID NOS.  
4 14-20, SEQ ID NOS. 23-24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO.  
5 32, SEQ ID NO. 34, and homologs and fragments thereof.

1           21. A method of detecting the presence of rickettsial nucleic acids comprising  
2 contacting a sample suspected of containing rickettsial nucleic acids with a composition  
3 comprising a labeled polynucleotide which encodes a polypeptide having the characteristic of  
4 eliciting an immune response protective against disease or death caused by a rickettsial  
5 pathogen, allowing for the formation of a hybridization complex and detecting said label.

1           22. The composition, according to claim 21, wherein said rickettsial pathogen is  
2 selected from the group consisting of *Rickettsia* spp., *Ehrlichia* spp., *Anaplasma* spp., and  
3 *Cowdria* spp.

1           23. The composition, according to claim 21, wherein said polypeptide has an amino acid  
2 sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6,  
3 SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NOS. 16-20, SEQ ID NO. 23, SEQ ID NO. 24, SEQ  
4 ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, and homologs  
5 and immunogenic fragments thereof.

1           24. The composition, according to claim 21, wherein said polynucleotide has a nucleic  
2 acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO.  
3 5, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NOS. 9-13, SEQ ID NO. 21, SEQ ID NO. 22, . SEQ

- 4 ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, homologs  
5 thereof, and fragments thereof which encode immunogenic polypeptides.

## FIG. 1A

<i>C.r.</i>	ATGAATTGCAAGAAAATTTTA-----TCACAAGTACACTAATAATCATTAGTG
<i>E.c.</i>	ATGAATTACAAAAAAGTTTCA-----TAACAGCG-ATTGATATCATTAATA
<i>A.m.</i>	ATGAATTACAGAGAAATTGTTTACAGGGGGCCTG-TCAGCAGCC-ACAGTCTGCGCCTGCT
	***** ** ** ** *
<i>C.r.</i>	TCATTTT--TACCTGGTGTCTCTTTTCTGATGTAATACAGGAAGACAGCAACCCAGCAG
<i>E.c.</i>	TCCTTCTCTTACCTGGAGTATCATTTCCGACCCCAAGCAGGTAGGTCA---TTAACG
<i>A.m.</i>	CCCTACTTGTAGTGGGCGCGTAGTGGCATCTCCCATGAGTCACGAAAGTGGCTTCTGAAG
	* * * * * ** * * * *
<i>C.r.</i>	GCAGTGTTTACATTAGCGCAAAATACATGCCAACTGCATCACATTTTGGTAAAATGTCAA
<i>E.c.</i>	GTAATTTCTACATCAGTGGAAAAATACGATGCCAAGGCTTCGCATTTTGGAGTATCTCTG
<i>A.m.</i>	GGGGAGTAATGGGAGGTAGCTTTTACGTGGGTGGGCCT-ACAGCCAGCATTTCCCTTCT
	* * * * * ** * * * *
<i>C.r.</i>	TCAAAGAAGATTCAAAAAATACTCAAACGGTATTTGGTCTAAAAAAGATTGGGATGGCG
<i>E.c.</i>	CTAAGGAAGAAAGAAATACAACAGTTGGAGTGTTTGGACTGAAGCAAAATTGGGACGGAA
<i>A.m.</i>	GTACCTCGTTTCGACATGCGTGAGTCAAGCAAAAGAGACCTCA--TACGTTAGAGGCTATG
	* * * * * * * * * *
<i>C.r.</i>	TTAAACACCATCAGATTCTAGCAATACTAAATTCACAAATTTTACTGAAAAAGACTATT
<i>E.c.</i>	GGCAATATC--CAACTCCTCCCCAAACGA-----TGATTTCACTGTCTCAAAATTATT
<i>A.m.</i>	ACAAGAGCATTGCAACGATTGATGTGAGTGTGCCAGCAAACTTTTCCAAATCTGGCTACA
	* ** * * * * ** *
<i>C.r.</i>	CTTTCAGATATGAAAAACAATCCGTTTTTAGGTTTTCGCTGGAGCAATTGGGTACTCAATGA
<i>E.c.</i>	CATTAAATATGAAAAACAACCCGTTTTTAGGTTTTGAGGCTATTGGTTACTCAATGG
<i>A.m.</i>	CTTTTGCCTTCTCTAAAAAACTTAATCACGTCCTTTCGACGGCGCTGTGGGATATCTCTGG
	* ** * * * * ** * * * *

## FIG. 1B

<i>C.r.</i>	ATGGACCAAGAAATAGAGTTCGAAGTATCCTATGAAACTTTTGATGTAAAAAACCTAGGTG	
<i>E.c.</i>	ATGGTCCCAAGAAATAGAGCTTGAAGTATCTTATGAAACATTTGATGTAAAAAATCAAGGTA	
<i>A.m.</i>	GAGGAGCCAGAGTGGAAATTGGAAGCGAGCTACAGAAAGTTTGCTACTTTGGCGGACGGGC	**
	** *	**
<i>C.r.</i>	GCAACTATAAAAACAACGCACACATGTACTGTGCTTTAGATACAGCAGCACAAATAGCA	
<i>E.c.</i>	ACAAATTATAAGAATGAAGCACATAGATATTGTGCTCTATCCCATAACTCAGCAGCAGACA	
<i>A.m.</i>	AGTACGCCAAAAAGTG-----GTGCGGAATCTCTGGCAGCTATTACCCGCG	*
	* *	*
<i>C.r.</i>	CTAATGGCGCAGGATTAACTACATCTGTTATGGTAAAAAACGAAATTTAACAAATATAT	
<i>E.c.</i>	TGAGTAGTGCAAG---TAATAATTGTTGCTTTCTAAAAAATGAAGGATTACTTGACATAT	
<i>A.m.</i>	ACGCTAACATTACTGAGACCAATTACTTCGTAGTCAAAAATTGATGAAATCACAACACCT	*
	* *	*
<i>C.r.</i>	CATTAAATGTTAAATGCGTGTATGATATCATGCTTGATGGAATACCAGTTTCTCCATATG	
<i>E.c.</i>	CATTATGCTGAACGCATGCTATGACGTAGTAGCGGAAGCATACCTTTTCTCCTTATA	
<i>A.m.</i>	CAGTCATGTTAAATGGCTGCTATGACGTGCTGCACACAGATTTACCTGTGTCCTCCGTATG	**
	** *	**
<i>C.r.</i>	TATGTGCAGGTATTGGCACTGACTTAGTGTGTCAGTAATTAATGCTACAAATCCTAAATTAT	
<i>E.c.</i>	TATGGCGCAGGTATCGGTACTGATTAGTATCCATGTTGAAGCTACAAATCCTAAATTT	
<i>A.m.</i>	TATGTCCGGGATAGCGCGAAGCTTTGTTGACATCTCTAAGCAAGTAACCAACAAGCTGG	***
	*** *	***
<i>C.r.</i>	CTTATCAAGGAAAGCTAGGCATAAGTTACTCAATCAATTCTGAAGCTTCTATTATCG	
<i>E.c.</i>	CTTACCAAGGAAAGTTAGGTTTAAGCTACTCTATTAAGCCAGAGCTTCTGTGTTATTG	
<i>A.m.</i>	CCTACAGGGGCAAGTTGGGATTAGCTACCAGTTTACTCCGGAAATATCCTTGGTGGCAG	*
	* *	*

## FIG. 1C

<i>C.r.</i>	GTGGACATTCCATAGAGTTATAGGTAATGAATTTAAAGATATTGCTACCTTAAATAATAT	
<i>E.c.</i>	GTGGGCACCTTTCATAAGGTAATAGGGAACGAATTTAGAGATATTCCTACTATAATACCTA	
<i>A.m.</i>	GTGGGTTCTACACGGGCTATTGTGATGAGTCTTACAAGGACATTCCCGCACACAACAGTG	
	*** * ** * * * * * * * * * *	
<i>C.r.</i>	TTACTTCAAAAACAGGAATATCTAATCCTGGCTTTGCATCAGCAACACTTGATGTTGTC	
<i>E.c.</i>	CTGGATCAACACTTGCAGGAAAGGAACTACCTGCAATAGTAATACTGGATGTATGCC	
<i>A.m.</i>	TAAAGTTCTCTGGAGAGCAAAA-----GCCTCAGTCAAAGCGCATATTGCTG	
	* * * * *	** * * *
<i>C.r.</i>	ACTTTGGTATAGAAAATTGGAGGAAGGTTTGTAATTTAA---	
<i>E.c.</i>	ACTTTGGAATAGAAAATGGGAGGAAGGTTTAA-----	
<i>A.m.</i>	ACTACGGCTTTAACCTTGGAGCAAGATTCCCTGTTTCAGCTAA	
	*** ** * * * **** ** *	

4/8

[illegible]

FIG. 2A

576

1341 tagcaaaatttagagcgatgcatcttttgggaactgaaccttgagggaagggttta tcttaaac  
V T L S V C H F G I E L G G R F N F \*

2401 ttctggttatgcgccatactgcttaaaaaatacctaacccttgctttatttcgtacagcaaac

2461 aaaaattagtgcgcacaaaagaatgtagcaatacaagaaccccccccccattacaattgctattct

2521 accatcacccccatttacaccacttacactcaaatcaacttgacaaatcacacacagcttcttggga

2581 aaaacaaaaciatactcttaaattctctcttacaaaaaacattttattctcttgactaaaaaacca  
-35

2641 gctttataaaccttgcttttttttcatttgtagctcttactatctgttaaccttttttccctattcttag  
-10

2701 gtgcacactgaatttgcacaaaaatttttatatcaaacactacattagttatgcgttaattgctctt  
RBS M N C K K F F I T T T L V S L M S P

2761 cttaccttggaatactcattttttgatgcagtagacagaacgacaaatcttggttggttaattctta  
L P G I S F S D A V Q N D N V G G N N F Y

2821 tactagtggggaaatatgtaccaagctgtttccacatttttggcggtattctcttgctaaacagga  
I S G K Y V P S V S H F G V F S A K Q E

2881 aagaaatacaacaacttcgggagtatcttggaattaagcaagaattgggtaggcagcacatact  
R N T T C I G V F G L K Q D W D G S T I S

2941 caaaaaattctctcggaaaatacattttaacgtctccaacacattctcaaaaacatcaaaaataa  
K N S P E N T T F N V P N Y S F K Y E N N

3001 tccattttctagggtttctcaggagctgttggtttcttaactgaatggtctcaagaatacagctt  
P P L G T P A G A V G Y L M N G P R I E L

3061 agaaatgtcttgaacacatttgatgtgaaaaaacccgggttaataactatgaagaacgatgc  
E M S Y E T F D V K N Q G N N Y K N D A

3121 tcscaaatatattatgcttttaacccataacagctgggggaaagcttaagcaatgcagggtgataa  
H K Y Y A A L T H N S G G K L S N A G D K

3181 gttctgttttttaaaaaattgaaggactactgatacttactttatgttggaatgcattgcta  
F V F L K N E G L L D I S L M L N A C Y

3241 tgatgtaataagtgaaaggaaacacottttttttttacatactgtgcagggtgttggtcactga  
D V I S E G I P F S P Y I C A G V G T D

3301 ttttaatatccatctctcgaaccccaaacgttaaaaattttttatcaaggaaagcttaggtttt  
L I S M F E A I N P K I S Y Q G K L G L

3361 gagttactccataagccctagaagctttctgttttttggttggaactttttataagggtgat  
S Y S I S P E A S V F V G G H F H K V I

3421 agggaaatgaatttcagagatatctcttggtatgataccccagctacctcaactctcacaggtaa  
G N E F R D I P A M I P S T S T L T G N

3481 tcaactttactatagtaacataagctgtatgccacttttggaagggaacttggaaggaggtt  
H F T I V T L S V C H F G V E L G G R F

3541 taacttttaatttttatttctgcccacatggttaaaaaataacttcaaaacttgtttttattattg  
N P I \*

3601 ctgcaggtaaatataaaattagtgcccaaaaagaatgtagcaataacagccccccccccactag  
3661 ttataaagctgctgttttttttaccctttacacatgatactataacttaaccaggtttttttgc  
3721 tactactttacctgacgttaacataataaaattttttttacaaaagcttagccgatactttatatac  
-35

3781 aaaaattttatactcttgactttgcttttatatgacacctctactattgttcaatttactttgct  
-10

3841 actatttagttatataatgaattacaaaaaaagttttcatacaacagtcattgatatcatta  
RBS M N Y K K V F I T S A L I S L

3901 atatctctctctacgttgaggtattctttccgacccagcaggttagtggtatttaacggttaatt  
I S S L P G V S F S D P A G S G I N G N

3961 tttcacatcagtcggaaaaatcacatgccaaagtgccttggcatttttgaggtattctctggcttaag  
F Y I S G K Y M P S A S H F G V F S A K

4021 gaagaaagaaatacaacagcttggaagtgttggtactgaagcgaataattgggaacggaagcgca  
E E R N T T V G V F G L K Q N W D G S A

4081 atatccaactctctcccaaacgatgtaattcactgtcttcaaaattatcccttttaaaatctcaaa  
I S N S S P N D V F T V S N Y S F K Y E

4141 aacaaacccgttttttaggtttctcaggagctatttggttaacttaactggatgcctcaagaata  
N N P F L G F A G A I G Y S M D G P R I

4201 gagcttgaagtattctatgaacacatttgatgtcaaaaaatacagggttaacaattataagaat  
E L E V S Y E T F D V K N Q G N N Y K N

4261 gaagccatagatatattgtgctcttatccataaaccaagcagcagacattgtagtgcgaagt  
E A H R Y C A L S H N S A A D M S S A S

4321 aataacttttcttttaaaaaattgaaggatttcttgacatactatttatgctgaacgca  
N N P V F L K N E G L L D I S F M L N A

4381 tgcattgacgttagtaggggaaggcaataccttttttttttatataagccagggtatcgggt  
C Y D V V G E G I P F S P Y I C A A G I G

4441 acctgttttagtatccatttgcaccccaaaaactgttaaaaatttttttaccagaaggaaagtta  
T D L V S M F E A T N P K I S Y Q G K L

4501 ggttttaagctactctataagccccaagaagctttctgttttcttggttggtgcactttttataag  
G L S Y S I S P E A S V F I G G H F H K

4561 gtaataagggaacgaatttagagatatctctactataataccctactggatcaaacactttgca  
Y I G N E F R D I P T I I P T G S T L A

4621 ggaaagggaacactaccctgcaatagtaatactgggaactgttggaactttgggaactgaagaatg  
G K G N Y P A I V I L D V C H F G I E M

4681 gga  
G

FIG. 2B

```

1  gggtgtaaatatgaatatcaaaaaaactttttacagtaactgcatagttattatttaacttc
   RBS      M K Y K K T F T V T A L V L L T S
51  ctttacacatctttatcacctttttatagttccagcagtgccagtgacaattcacaactttta
   F T H F I P F Y S P A R A S T I H N F Y
121 catttagtggaaaatcatatgcccacagcgtcacatcttttggaaatttttttagctaaagaaga
   I S G K Y M P T A S H F G I F S A K E E
181 acaaagtttttactaaaggtatttagttgggttagattcaacgatttatcacataactattatcaa
   Q S F T K V L V G L D Q R L S H N I I N
241 caataatgatacagcaaaagagtttttaaggtttcaaaattattttatataaaatcaaaaaataa
   N N D T A K S L K V Q N Y S F K Y K N N
301 cccatttttaggattttgcaggagcttattgggttattcaataggcattttcaagaatagaact
   P F L G F A G A I G Y S I G N S R I E L
361 agaagttatcacatgaatactttgatactaaaaacccagggaacaactattttaaatgacttc
   E V S H E I F D T K N P G N N Y L N D S
421 ccacaaaatattgcgcttttatctcattggaagttcacatattgcagtgatggaaatagcggaga
   H K Y C A L S H G S H I C S D G N S G D
481 ttggtacactgcaaaaaactgataagttttgtactttttgaaaaatgaaggttttactttgacgt
   W Y T A K T D K P V L L K N E G L L D V
541 ctcattttatgttaaacgcattgtttatgacataaaccaactgaaaaaattgcctttttcacctta
   S F M L N A C Y D I T T E K M P F S P Y
601 tatatgtgcaggtatttgggtactgtatttcatactttttgtttgagacaacacaaaaacaaaaa
   I C A G I G T D L I S M F E T T Q N K I
661 atctttatcaagggaaggttaggttttaacttatctataaactcaagagctttttgtttttgc
   S Y Q G K L G L N Y T I N S R V S V F A
721 aggtgggcacttttcataaggttaattaggttaattgaatttttaaggttatttcctacttttattcc
   G G H F H K V I G N E F K G I P T L L P
781 tgatggatcaaacattaaagtcacaacagttcttgaacagtaacatttagatgtgtgcccatttc
   D G S N I K V Q Q S A T V T L D V C H F
841 cgggttagagattggaagtagatttttttttaatactttttattgtacatgttaaaaaata
   G L E I G S R F F F *
901 gtacttagtttgccttctgtgctttataaaacgcagagagaaaatagtttagtaataaaattaga
961 aagttaaaatttagaaaaagtcattatgttttttttactgttcatttgatactcaactcaaaaagtag
1021 tataaatgttactttatttaataatttttacgtagctattttaaattttcccttacaaaaagccac
1081 tagtatttttatactaaaaagcttatacttttgggtttgattttaattgtatttttactactgt
   -35      -10
1141 caattttactttcactgttttttgggtgtaaatatgaactgcaaaaaagtttttcacaataagt
   RBS      M N C K K V F T I S
1201 gcattgatatccatccatatacttttacttaattgttttcatacttcaacccagttatattggt
   A L I S S I Y F L P N V S Y S N P V Y G
1261 aacagttatgtatggtaatttttacatattcagggaagtagcatgccaagtggttcttcatttt
   N S M Y G N F Y I S G K Y M P S V P H F
1321 ggaatttttttcagctgaagaagagaaaaaaaagacactgttagttatattggcttaaaagaa
   G I F S A E E E K K K T T V V Y G L K E
1381 aactgggcaggagatgcaatatcttagtcaaaagtccagatgataattttaccatttcgaaat
   N W A G D A I S S Q S P D D N F T I R N
1441 tactttattcaagttatgcaagcaacaagttttttaggttttgcagtagctattgtgttacttg
   Y S F K Y A S N K F L G F A V A I G Y S
1501 ataggcagttccaaagaatagaagttggagatgtttttatgaagcatttgatgtcaaaaaatcaa
   I G S P R I E V E M S Y E A F D V K N Q
1561 ggtaacaatt
      G N N

```

FIG. 2C

1 acatgtatacattatagtaacaaatggttaccgatttttattcataagttaagtaaaaatct  
61 ataccattctcttttcacttttatcagaagactttttatttatcaciaaactcatgacgtatag  
121 tgtcacaaaataaacacactgcaactgcaatcactacgtaaaaacttttaactcttctttttt  
181 acaactaaaatacttaataaaaagtaatatagttataaaaaatctttaagtaacTTGACAtaat  
-35  
241 attactctgataTAGCATatgtctagttatctctataactaaacgtttatataattGGAGCa  
-10  
301 tattaATGAAAGCTATCAAATTCATACTTAATGTCTGCTTACTATTTGCAGCAATATTTT  
M K A I K F I L N V C L L F A → A I F L  
361 TAGGGTATTCCTATATTACAAAACAAGGCATATTTCAAACAAAACATCATGATACACCTA  
G Y S Y I T K Q G I F Q T K H H D T P N  
421 ATACTACTATACCAAATGAAGACGGTATTCATCTAGCTTTAGCTTAATCAATCAAGACG  
T T I P N E D G I Q S S F S L I N Q D G  
481 GTAAAACAGTAACCAGCCAAGATTTCTTAGGGAAACACATGTTAGTTTTGTTTGGATTCT  
K T V T S Q D F L G K H M L V L F G F S  
541 CTGCATGTAAAAGCATTGCCCCTGCAGAAATTGGGATTAGTATCTGAAGCACTTGCAACAAC  
A C K S I C P A E L G L V S E A L A Q L  
601 TTGGTAATAATGCAGACAAATTACAAGTAATTTTTATTACAATTGATCCAAAAAATGATA  
G N N A D K L Q V I F I T I D P K N D T  
661 CTGTAGAAAAATTAAAGAATTTTCATGAACATTTTGATTCAAGAATTCAAATGTTAACAG  
V E K L K E F H E H F D S R I Q M L T G  
721 GAAATACTGAAGACATTAATCAAATAATTAAAAATTATAAAATATATGTTGGACAAGCAG  
N T E D I N Q I I K N Y K I Y V G Q A D  
781 ATAAAGATCATCAAATTAACCATTCTGCAATATGTACCTTATTGACAAAAAAGGATCAT  
K D H Q I N H S A I M Y L I D K K G S Y  
841 ATCTTTCACACTTCATTCCAGATTTAAAAATCACAAGAAAATCAAGTAGATAAGTTACTAT  
L S H F I P D L K S Q E N Q V D K L L S  
901 CTTTAGTTAAGCAGTATCTGTAATttaataattaattAAAGagaatagttacacaCTTTtt  
L V K Q Y L \*  
961 ataaattcatggaatacgttggttggttaggttttttttagtatttttagtgcttaataac  
1021 attggcat

FIG. 3A

FIG. 3B

## SEQUENCE LISTING

<110> University of Florida

<120> Nucleic Acid Vaccines Against Rickettsial Diseases and  
Methods of use

<130> UF-167C3

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<150> 08/953,326

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   1             5             10             15

tca ttt tta cct ggt gtg tcc ttt tct gat gta ata cag gaa gac agc   96
Ser Phe Leu Pro Gly Val Ser Phe Ser Asp Val Ile Gln Glu Asp Ser
           20             25             30

aac cca gca ggc agt gtt tac att agc gca aaa tac atg cca act gca   144
Asn Pro Ala Gly Ser Val Tyr Ile Ser Ala Lys Tyr Met Pro Thr Ala
           35             40             45

tca cat ttt ggt aaa atg tca atc aaa gaa gat tca aaa aat act caa   192
Ser His Phe Gly Lys Met Ser Ile Lys Glu Asp Ser Lys Asn Thr Gln
           50             55             60

acg gta ttt ggt cta aaa aaa gat tgg gat ggc gtt aaa aca cca tca   240
Thr Val Phe Gly Leu Lys Lys Asp Trp Asp Gly Val Lys Thr Pro Ser
           65             70             75             80

gat tct agc aat act aat tct aca att ttt act gaa aaa gac tat tct   288

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2

Asp	Ser	Ser	Asn	Thr	Asn	Ser	Thr	Ile	Phe	Thr	Glu	Lys	Asp	Tyr	Ser		
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ttc	aga	tat	gaa	aac	aat	ccg	ttt	tta	ggt	ttc	gct	gga	gca	att	ggg	336	
Phe	Arg	Tyr	Glu	Asn	Asn	Pro	Phe	Leu	Gly	Phe	Ala	Gly	Ala	Ile	Gly		
			100					105					110				
tac	tca	atg	aat	gga	cca	aga	ata	gag	ttc	gaa	gta	tcc	tat	gaa	act	384	
Tyr	Ser	Met	Asn	Gly	Pro	Arg	Ile	Glu	Phe	Glu	Val	Ser	Tyr	Glu	Thr		
			115				120					125					
ttt	gat	gta	aaa	aac	cta	ggt	ggc	aac	tat	aaa	aac	aac	gca	cac	atg	432	
Phe	Asp	Val	Lys	Asn	Leu	Gly	Gly	Asn	Tyr	Lys	Asn	Asn	Ala	His	Met		
			130			135					140						
tac	tgt	gct	tta	gat	aca	gca	gca	caa	aat	agc	act	aat	ggc	gca	gga	480	
Tyr	Cys	Ala	Leu	Asp	Thr	Ala	Ala	Gln	Asn	Ser	Thr	Asn	Gly	Ala	Gly		
			145			150				155					160		
tta	act	aca	tct	gtt	atg	gta	aaa	aac	gaa	aat	tta	aca	aat	ata	tca	528	
Leu	Thr	Thr	Ser	Val	Met	Val	Lys	Asn	Glu	Asn	Leu	Thr	Asn	Ile	Ser		
				165					170					175			
tta	atg	tta	aat	gcg	tgt	tat	gat	atc	atg	ctt	gat	gga	ata	cca	gtt	576	
Leu	Met	Leu	Asn	Ala	Cys	Tyr	Asp	Ile	Met	Leu	Asp	Gly	Ile	Pro	Val		
			180					185					190				
tct	cca	tat	gta	tgt	gca	ggt	att	ggc	act	gac	tta	gtg	tca	gta	att	624	
Ser	Pro	Tyr	Val	Cys	Ala	Gly	Ile	Gly	Thr	Asp	Leu	Val	Ser	Val	Ile		
			195				200					205					
aat	gct	aca	aat	cct	aaa	tta	tct	tat	caa	gga	aag	cta	ggc	ata	agt	672	
Asn	Ala	Thr	Asn	Pro	Lys	Leu	Ser	Tyr	Gln	Gly	Lys	Leu	Gly	Ile	Ser		
			210			215					220						
tac	tca	atc	aat	tct	gaa	gct	tct	atc	ttt	atc	ggt	gga	cat	ttc	cat	720	
Tyr	Ser	Ile	Asn	Ser	Glu	Ala	Ser	Ile	Phe	Ile	Gly	Gly	His	Phe	His		
					225		230			235				240			
aga	gtt	ata	ggt	aat	gaa	ttt	aaa	gat	att	gct	acc	tta	aaa	ata	ttt	768	
Arg	Val	Ile	Gly	Asn	Glu	Phe	Lys	Asp	Ile	Ala	Thr	Leu	Lys	Ile	Phe		
				245				250						255			
act	tca	aaa	aca	gga	ata	tct	aat	cct	ggc	ttt	gca	tca	gca	aca	ctt	816	
Thr	Ser	Lys	Thr	Gly	Ile	Ser	Asn	Pro	Gly	Phe	Ala	Ser	Ala	Thr	Leu		
				260				265					270				
gat	gtt	tgt	cac	ttt	ggt	ata	gaa	att	gga	gga	agg	ttt	gta	ttt	taa	864	
Asp	Val	Cys	His	Phe	Gly	Ile	Glu	Ile	Gly	Gly	Arg	Phe	Val	Phe			
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&lt;400&gt; 2

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Ser Phe Leu Pro Gly Val Ser Phe Ser Asp Val Ile Gln Glu Asp Ser
      20              25              30

Asn Pro Ala Gly Ser Val Tyr Ile Ser Ala Lys Tyr Met Pro Thr Ala
      35              40              45

Ser His Phe Gly Lys Met Ser Ile Lys Glu Asp Ser Lys Asn Thr Gln
      50              55              60

Thr Val Phe Gly Leu Lys Lys Asp Trp Asp Gly Val Lys Thr Pro Ser
      65              70              75              80

Asp Ser Ser Asn Thr Asn Ser Thr Ile Phe Thr Glu Lys Asp Tyr Ser
      85              90              95

Phe Arg Tyr Glu Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly
      100              105              110

Tyr Ser Met Asn Gly Pro Arg Ile Glu Phe Glu Val Ser Tyr Glu Thr
      115              120              125

Phe Asp Val Lys Asn Leu Gly Gly Asn Tyr Lys Asn Asn Ala His Met
      130              135              140

Tyr Cys Ala Leu Asp Thr Ala Ala Gln Asn Ser Thr Asn Gly Ala Gly
      145              150              155              160

Leu Thr Thr Ser Val Met Val Lys Asn Glu Asn Leu Thr Asn Ile Ser
      165              170              175

Leu Met Leu Asn Ala Cys Tyr Asp Ile Met Leu Asp Gly Ile Pro Val
      180              185              190

Ser Pro Tyr Val Cys Ala Gly Ile Gly Thr Asp Leu Val Ser Val Ile
      195              200              205

Asn Ala Thr Asn Pro Lys Leu Ser Tyr Gln Gly Lys Leu Gly Ile Ser
      210              215              220

Tyr Ser Ile Asn Ser Glu Ala Ser Ile Phe Ile Gly Gly His Phe His
      225              230              235              240

Arg Val Ile Gly Asn Glu Phe Lys Asp Ile Ala Thr Leu Lys Ile Phe
      245              250              255

Thr Ser Lys Thr Gly Ile Ser Asn Pro Gly Phe Ala Ser Ala Thr Leu
      260              265              270

Asp Val Cys His Phe Gly Ile Glu Ile Gly Gly Arg Phe Val Phe

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280

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ctt ctc tta cct gga gta tca ttt tcc gac cca agg cag gta gtg gtc 96  
 Leu Leu Leu Pro Gly Val Ser Phe Ser Asp Pro Arg Gln Val Val Val  
 20 25 30

att aac ggt aat ttc tac atc agt gga aaa tac gat gcc aag gct tcg 144  
 Ile Asn Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Asp Ala Lys Ala Ser  
 35 40 45

cat ttt gga gta ttc tct gct aag gaa gaa aga aat aca aca gtt gga 192  
 His Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly  
 50 55 60

gtg ttt gga ctg aag caa aat tgg gac gga agc gca ata tcc aac tcc 240  
 Val Phe Gly Leu Lys Gln Asn Trp Asp Gly Ser Ala Ile Ser Asn Ser  
 65 70 75 80

tcc cca aac gat gta ttc act gtc tca aat tat tca ttt aaa tat gaa 288  
 Ser Pro Asn Asp Val Phe Thr Val Ser Asn Tyr Ser Phe Lys Tyr Glu  
 85 90 95

aac aac ccg ttt tta ggt ttt gca gga gct att ggt tac tca atg gat 336  
 Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp  
 100 105 110

ggt cca aga ata gag ctt gaa gta tct tat gaa aca ttt gat gta aaa 384  
 Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Thr Phe Asp Val Lys  
 115 120 125

aat caa ggt aac aat tat aag aat gaa gca cat aga tat tgt gct cta 432  
 Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Cys Ala Leu  
 130 135 140

tcc cat aac tca gca gca gac atg agt agt gca agt aat aat ttt gtc 480  
 Ser His Asn Ser Ala Ala Asp Met Ser Ser Ala Ser Asn Asn Phe Val  
 145 150 155 160

ttt cta aaa aat gaa gga tta ctt gac ata tca ttt atg ctg aac gca 528  
 Phe Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Phe Met Leu Asn Ala

5

165	170	175	
tgc tat gac gta gta ggc gaa ggc	ata cct ttt tct cct tat ata tgc		576
Cys Tyr Asp Val Val Gly Glu Gly	Ile Pro Phe Ser Pro Tyr Ile Cys		
180	185	190	
gca ggt atc ggt act gat tta gta tcc atg ttt gaa gct aca aat cct			624
Ala Gly Ile Gly Thr Asp Leu Val Ser Met Phe Glu Ala Thr Asn Pro			
195	200	205	
aaa att tct tac caa gga aag tta ggt tta agc tac tct ata agc cca			672
Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro			
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gaa gct tct gtg ttt att ggt ggg cac ttt cat aag gta ata ggg aac			720
Glu Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn			
225	230	235	240
gaa ttt aga gat att cct act ata ata cct act gga tca aca ctt gca			768
Glu Phe Arg Asp Ile Pro Thr Ile Ile Pro Thr Gly Ser Thr Leu Ala			
245	250		255
gga aaa gga aac tac cct gca ata gta ata ctg gat gta tgc cac ttt			816
Gly Lys Gly Asn Tyr Pro Ala Ile Val Ile Leu Asp Val Cys His Phe			
260	265	270	
gga ata gaa atg gga gga agg ttt aa			842
Gly Ile Glu Met Gly Gly Arg Phe			
275	280		

&lt;210&gt; 4

&lt;211&gt; 280

&lt;212&gt; PRT

&lt;213&gt; Ehrlichia chaffeensis

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Met Asn Tyr Lys Lys Ser Phe Ile Thr Ala Ile Asp Ile Ile Asn Ile
1 5 10 15

Leu Leu Leu Pro Gly Val Ser Phe Ser Asp Pro Arg Gln Val Val Val
20 25 30

Ile Asn Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Asp Ala Lys Ala Ser
35 40 45

His Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly
50 55 60

Val Phe Gly Leu Lys Gln Asn Trp Asp Gly Ser Ala Ile Ser Asn Ser
65 70 75 80

Ser Pro Asn Asp Val Phe Thr Val Ser Asn Tyr Ser Phe Lys Tyr Glu
85 90 95

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Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp
      100                      105                      110

Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Thr Phe Asp Val Lys
      115                      120                      125

Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Cys Ala Leu
      130                      135                      140

Ser His Asn Ser Ala Ala Asp Met Ser Ser Ala Ser Asn Asn Phe Val
      145                      150                      155                      160

Phe Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Phe Met Leu Asn Ala
      165                      170                      175

Cys Tyr Asp Val Val Gly Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys
      180                      185                      190

Ala Gly Ile Gly Thr Asp Leu Val Ser Met Phe Glu Ala Thr Asn Pro
      195                      200                      205

Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro
      210                      215                      220

Glu Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn
      225                      230                      235                      240

Glu Phe Arg Asp Ile Pro Thr Ile Ile Pro Thr Gly Ser Thr Leu Ala
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Gly Lys Gly Asn Tyr Pro Ala Ile Val Ile Leu Asp Val Cys His Phe
      260                      265                      270

Gly Ile Glu Met Gly Gly Arg Phe
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tgcc gcc tgc tcc cta ctt gtt agt ggg gcc gta gtg gca tct ccc atg	96
Cys Ala Cys Ser Leu Leu Val Ser Gly Ala Val Val Ala Ser Pro Met	
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agt cac gaa gtg gct tct gaa ggg gga gta atg gga ggt agc ttt tac	144
Ser His Glu Val Ala Ser Glu Gly Gly Val Met Gly Gly Ser Phe Tyr	
35 40 45	
gtg ggt gcg gcc tac agc cca gca ttt cct tct gtt acc tcg ttc gac	192
Val Gly Ala Ala Tyr Ser Pro Ala Phe Pro Ser Val Thr Ser Phe Asp	
50 55 60	
atg cgt gag tca agc aaa gag acc tca tac gtt aga ggc tat gac aag	240
Met Arg Glu Ser Ser Lys Glu Thr Ser Tyr Val Arg Gly Tyr Asp Lys	
65 70 75 80	
agc att gca acg att gat gtg agt gtg cca gca aac ttt tcc aaa tct	288
Ser Ile Ala Thr Ile Asp Val Ser Val Pro Ala Asn Phe Ser Lys Ser	
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Gly Tyr Thr Phe Ala Phe Ser Lys Asn Leu Ile Thr Ser Phe Asp Gly	
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gct gtg gga tat tct ctg gga gga gcc aga gtg gaa ttg gaa gcg agc	384
Ala Val Gly Tyr Ser Leu Gly Gly Ala Arg Val Glu Leu Glu Ala Ser	
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tac aga agg ttt gct act ttg gcg gac ggg cag tac gca aaa agt ggt	432
Tyr Arg Arg Phe Ala Thr Leu Ala Asp Gly Gln Tyr Ala Lys Ser Gly	
130 135 140	
gcg gaa tct ctg gca gct att acc cgc gac gct aac att act gag acc	480
Ala Glu Ser Leu Ala Ala Ile Thr Arg Asp Ala Asn Ile Thr Glu Thr	
145 150 155 160	
aat tac ttc gta gtc aaa att gat gaa atc aca aac acc tca gtc atg	528
Asn Tyr Phe Val Val Lys Ile Asp Glu Ile Thr Asn Thr Ser Val Met	
165 170 175	
tta aat ggc tgc tat gac gtg ctg cac aca gat tta cct gtg tcc ccg	576
Leu Asn Gly Cys Tyr Asp Val Leu His Thr Asp Leu Pro Val Ser Pro	
180 185 190	
tat gta tgt gcc ggg ata ggc gca agc ttt gtt gac atc tct aag caa	624
Tyr Val Cys Ala Gly Ile Gly Ala Ser Phe Val Asp Ile Ser Lys Gln	
195 200 205	
gta acc aca aag ctg gcc tac agg ggc aag gtt ggg att agc tac cag	672
Val Thr Thr Lys Leu Ala Tyr Arg Gly Lys Val Gly Ile Ser Tyr Gln	
210 215 220	

8

ttt act ccg gaa ata tcc ttg gtg gca ggt ggg ttc tac cac ggg cta 720  
 Phe Thr Pro Glu Ile Ser Leu Val Ala Gly Gly Phe Tyr His Gly Leu  
 225 230 235 240

ttt gat gag tct tac aag gac att ccc gca cac aac agt gta aag ttc 768  
 Phe Asp Glu Ser Tyr Lys Asp Ile Pro Ala His Asn Ser Val Lys Phe  
 245 250 255

tct gga gaa gca aaa gcc tca gtc aaa gcg cat att gct gac tac ggc 816  
 Ser Gly Glu Ala Lys Ala Ser Val Lys Ala His Ile Ala Asp Tyr Gly  
 260 265 270

ttt aac ctt gga gca aga ttc ctg ttc agc taa 849  
 Phe Asn Leu Gly Ala Arg Phe Leu Phe Ser  
 275 280

&lt;210&gt; 6

&lt;211&gt; 282

&lt;212&gt; PRT

&lt;213&gt; Anaplasma marginale

&lt;400&gt; 6

Met Asn Tyr Arg Glu Leu Phe Thr Gly Gly Leu Ser Ala Ala Thr Val  
 1 5 10 15

Cys Ala Cys Ser Leu Leu Val Ser Gly Ala Val Val Ala Ser Pro Met  
 20 25 30

Ser His Glu Val Ala Ser Glu Gly Gly Val Met Gly Gly Ser Phe Tyr  
 35 40 45

Val Gly Ala Ala Tyr Ser Pro Ala Phe Pro Ser Val Thr Ser Phe Asp  
 50 55 60

Met Arg Glu Ser Ser Lys Glu Thr Ser Tyr Val Arg Gly Tyr Asp Lys  
 65 70 75 80

Ser Ile Ala Thr Ile Asp Val Ser Val Pro Ala Asn Phe Ser Lys Ser  
 85 90 95

Gly Tyr Thr Phe Ala Phe Ser Lys Asn Leu Ile Thr Ser Phe Asp Gly  
 100 105 110

Ala Val Gly Tyr Ser Leu Gly Gly Ala Arg Val Glu Leu Glu Ala Ser  
 115 120 125

Tyr Arg Arg Phe Ala Thr Leu Ala Asp Gly Gln Tyr Ala Lys Ser Gly  
 130 135 140

Ala Glu Ser Leu Ala Ala Ile Thr Arg Asp Ala Asn Ile Thr Glu Thr  
 145 150 155 160

Asn Tyr Phe Val Val Lys Ile Asp Glu Ile Thr Asn Thr Ser Val Met  
 165 170 175

Leu Asn Gly Cys Tyr Asp Val Leu His Thr Asp Leu Pro Val Ser Pro  
 180 185 190  
 Tyr Val Cys Ala Gly Ile Gly Ala Ser Phe Val Asp Ile Ser Lys Gln  
 195 200 205  
 Val Thr Thr Lys Leu Ala Tyr Arg Gly Lys Val Gly Ile Ser Tyr Gln  
 210 215 220  
 Phe Thr Pro Glu Ile Ser Leu Val Ala Gly Gly Phe Tyr His Gly Leu  
 225 230 235 240  
 Phe Asp Glu Ser Tyr Lys Asp Ile Pro Ala His Asn Ser Val Lys Phe  
 245 250 255  
 Ser Gly Glu Ala Lys Ala Ser Val Lys Ala His Ile Ala Asp Tyr Gly  
 260 265 270  
 Phe Asn Leu Gly Ala Arg Phe Leu Phe Ser  
 275 280

<210> 7  
 <211> 132  
 <212> DNA  
 <213> Ehrlichia chaffeensis

<400> 7  
 ggaatgaatt cagggacatt tctactctta aagcgtttgc tacaccatca tctgcagcta 60  
 ctccagactt agcaacagta aacttgagtg tgtgtcactt tggagtagaa cttggaggaa 120  
 gatttaactt ct 132

<210> 8  
 <211> 861  
 <212> DNA  
 <213> Ehrlichia chaffeensis

<400> 8  
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 gtggaaagta tatgccaagc gcttcgcatt ttggagtttt ttctgccaag gaagaaagaa 180  
 atacaacagt tggagtattt ggaatagagc aagattggga tagatgtgta atatctagaa 240  
 ccactttaag cgatatatto accgttccaa attattcatt taagtatgaa aataatctat 300  
 tttcaggatt tgcaggagct attggctact caatggatgg cccaagaata gagcttgaag 360  
 tatcttatga agcattcgat gttaaaaatc aaggtaacaa ttataagaac gaagcacata 420

gatattatgc tctgtcccat cttctcggca cagagacaca gatagatggg gcaggcagtg 480  
cgtctgtctt tctaataaat gaaggactac ttgataaatc atttatgctg aacgcatgtt 540  
atgatgtaat aagtgaaggg ataccctttt ctccttatat atgtgcaggt attggtattg 600  
atttagtata catgtttgaa gctataaatc ctaaaatttc ttatcaagga aaattagggt 660  
taagttaccc tataagccca gaagcttctg tgtttattgg tggacatttt cataaggtga 720  
taggaaacga atttagagat attcctacta tgatacctag tgaatcagcg cttgcaggaa 780  
aaggaaacta ccttgcaata gtaacactgg acgtgttcta ctttggcata gaacttggag 840  
gaaggtttaa cttccaactt t 861

&lt;210&gt; 9

&lt;211&gt; 837

&lt;212&gt; DNA

&lt;213&gt; Ehrlichia chaffeensis

&lt;400&gt; 9

atatgaattg caaaaaattt ttataacaa ctgcattagt atcactaatg tcctttctac 60  
ctggaatata attttctgat ccagtgcaag gtgacaatat tagtggtaat ttctatgtta 120  
gtggcaagta tatgccaagt gcttcgcatt ttggcatgtt ttctgccaaa gaagaaaaaa 180  
atcctactgt tgcattgtat ggcttaaaac aagattggga agggattagc tcatcaagtc 240  
acaatgataa tcatttcaat aacaagggtt attcatttaa atatgaaaat aaccattttt 300  
tagggtttgc aggagctatt ggttattcaa tgggtgggtc aagagtagag tttgaagtgt 360  
cctatgaaac atttgacgtt aaaaatcagg gtaataacta taaaaatgat gctcacagat 420  
actgtgcttt aggtcaacaa gacaacagcg gaataoctaa aactagtaaa tacgtactgt 480  
taaaaagcga aggattgctt gacatatcat ttatgctaaa tgcattgctat galataataa 540  
acgagagcat acccttgtct cttacatat gtgcagggtg tggactgat ttaatatcca 600  
tgtttgaagc tacaaatcct aaaatttctt accaaggga gttagggtcta agttactcta 660  
taaaccaga agcttctgta ttatttggtg gacattttca taagggtgata ggaaacgaat 720  
ttagggacat tcctactctg aaagcatttg ttacgtcacc agctactcca gatctagcaa 780  
tagtaacact aagtgtatgt ctttttggaa tagaacttgg aggaagggtt aacttct 837

&lt;210&gt; 10

&lt;211&gt; 843

&lt;212&gt; DNA

<213> Ehrlichia chaffeensis

<400> 10

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ctgggaatata attttctgat gcagtacaga acgacaattg tgggtggtaat ttctatatca 120
gtgggaaata tgtaccaagt gtttcacatt ttggcgattt ctctgctaaa caggaaagaa 180
atacaacaat cggagtatTTT ggattaaagc aagattggga tggcagcaca atatctaaaa 240
attctccaga aaatacatTTT aacgttccaa attattcatt taaatatgaa aataatccat 300
ttctaggTTT tgcaggagct gttgggttatt taatgaatgg tccaagaata gagttagaaa 360
tgtcctatga aacatttgat gtgaaaaacc agggtaataa ctataagaac gatgctcaca 420
aatattatgc ttttaacccat aacagtgggg gaaagctaag caatgcaggt gataagtttg 480
tttttctaaa aaatgaagga ctacttgata tatcacttat gttgaatgca tgctatgatg 540
taataagtga aggaatacct ttctctcctt acatatgtgc aggtgttggt actgatttaa 600
tatccatggt tgaagctata aaccctaaaa tttcttatca aggaaagtta ggtttgagtt 660
actccataag cccagaagct tctgtttttg ttgggtggaca ttttcataag gtgataggga 720
atgaattcag agatatctct gctatgatac ccagtacctc aactctcaca ggtaatcact 780
ttactatagt aacactaagt gtatgccact ttggagtgga acttgaggga aggtttaact 840
ttt 843

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<210> 11

<211> 830

<212> DNA

<213> Ehrlichia chaffeensis

<400> 11

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ctggagtatc attttccgac ccagcaggta gtgggtattaa cggtaatttc tacatcagtg 120
gaaaatacat gccaaagtgc tgcatttttg gagtattctc tgctaaggaa gaaagaaata 180
caacagttgg agtgtttgga ctgaagcaaa attgggacgg aagcgcaata tccaactcct 240
cccaaacga tgtattcact gtctcaaatt attcatttaa atatgaaaac aaccogtttt 300
taggttttgc aggagctatt ggttactcaa tggatgggtcc aagaatagag cttgaagtat 360
cttatgaaac atttgatgta aaaaatcaag gtaacaatta taagaatgaa gcacatagat 420
attgtgctct atcccataac tcagcagcag acatgagtag tgcaagtaat aattttgtct 480

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12

ttctaaaaaa tgaaggatta cttgacatat cattttatgct gaacgcatgc tatgacgtag 540  
 tagggogaagg catacctttt tctccttata tatgcgcagg tatcggtact gatttagtat 600  
 ccatgtttga agctacaaat cctaaaattt cttaccaagg aaagttaggt ttaagctact 660  
 ctataagccc agaagcttct gtgtttattg gtgggcactt tcataaggta atagggaacg 720  
 aatttagaga tattcctact ataataccta ctggatcaac acttgcagga aaaggaaaact 780  
 accctgcaat agtaatactg gatgtatgcc actttggaat agaaatggga 830

&lt;210&gt; 12

&lt;211&gt; 864

&lt;212&gt; DNA

&lt;213&gt; Ehrlichia canis

&lt;400&gt; 12

atatgaaata taaaaaaact ttacagtaa ctgcattagt attattaact tcttttacac 60  
 attttatacc tttttatagt ccagcacgtg ccagtacaat tcacaacttc tacattagt 120  
 gaaaatatat gccaacagcg tcacattttg gaattttttc agctaaagaa gaacaaagtt 180  
 ttactaaggt attagttggg ttagatcaac gattatcaca taatattata aacaataatg 240  
 atacagcaaa gagtcttaag gttcaaaatt attcatttaa atacaaaaat aaccatttc 300  
 taggatttgc aggagctatt ggttattcaa taggcaattc aagaatagaa ctagaagtat 360  
 cacatgaaat atttgatact aaaaaccag gaaacaatta tttaaagac tctcaciaat 420  
 attgcgcttt atctcatgga agtcacatat gcagtgatgg aaatagcgga gattggtaca 480  
 ctgcaaaaaac tgataagttt gtacttctga aaaatgaagg ttacttgac gtctcattha 540  
 tgttaaacgc atgttatgac ataacaactg aaaaaatgcc tttttcacct tatatatgtg 600  
 caggatttgg tactgatctc atatctatgt ttgagacaac acaaaacaaa atatcttate 660  
 aaggaaagtt aggtttaaac tatactataa actcaagagt ttctgttttc gcagggtgggc 720  
 actttcataa ggtaataggt aatgaattta aaggatttcc tactctatta cctgatggat 780  
 caaacattaa agtacaacag tctgcaacag taacattaga tgtgtgccat ttggggttag 840  
 agattggaag tagatttttc tttt 864

&lt;210&gt; 13

&lt;211&gt; 399

&lt;212&gt; DNA

&lt;213&gt; Ehrlichia canis

<400> 13  
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 ctaatgtctc atactctaac ccagtatatg gtaacagtat gtatggtaat ttttacatat 120  
 caggaaagta catgccaaagt gttcctcatt ttggaatttt ttcagctgaa gaagagaaaa 180  
 aaaagacaac tgtagtatat ggcttaaaaag aaaactgggc aggagatgca atatctagtc 240  
 aaagtcacaga tgataatttt accattcgaa attactcatt caagtatgca agcaacaagt 300  
 ttttaggggtt tgcagtagct attgggttact cgataggcag tccaagaata gaagttgaga 360  
 tgtcttatga agcatttgat gtaaaaaatc aaggtaaca 399

<210> 14  
 <211> 43  
 <212> PRT  
 <213> Ehrlichia chaffeensis

<400> 14  
 Asn Glu Phe Arg Asp Ile Ser Thr Leu Lys Ala Phe Ala Thr Pro Ser  
 1 5 10 15  
 Ser Ala Ala Thr Pro Asp Leu Ala Thr Val Thr Leu Ser Val Cys His  
 20 25 30  
 Phe Gly Val Glu Leu Gly Gly Arg Phe Asn Phe  
 35 40

<210> 15  
 <211> 286  
 <212> PRT  
 <213> Ehrlichia chaffeensis

<400> 15  
 Met Asn Cys Glu Lys Phe Phe Ile Thr Thr Ala Leu Thr Leu Leu Met  
 1 5 10 15  
 Ser Phe Leu Pro Gly Ile Ser Leu Ser Asp Pro Val Gln Asp Asp Asn  
 20 25 30  
 Ile Ser Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Ala Ser  
 35 40 45  
 His Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly  
 50 55 60  
 Val Phe Gly Ile Glu Gln Asp Trp Asp Arg Cys Val Ile Ser Arg Thr  
 65 70 75 80  
 Thr Leu Ser Asp Ile Phe Thr Val Pro Asn Tyr Ser Phe Lys Tyr Glu  
 85 90 95

14

Asn Asn Leu Phe Ser Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp  
 100 105 110  
 Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Ala Phe Asp Val Lys  
 115 120 125  
 Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Tyr Ala Leu  
 130 135 140  
 Ser His Leu Leu Gly Thr Glu Thr Gln Ile Asp Gly Ala Gly Ser Ala  
 145 150 155 160  
 Ser Val Phe Leu Ile Asn Glu Gly Leu Leu Asp Lys Ser Phe Met Leu  
 165 170 175  
 Asn Ala Cys Tyr Asp Val Ile Ser Glu Gly Ile Pro Phe Ser Pro Tyr  
 180 185 190  
 Ile Cys Ala Gly Ile Gly Ile Asp Leu Val Ser Met Phe Glu Ala Ile  
 195 200 205  
 Asn Pro Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Pro Ile  
 210 215 220  
 Ser Pro Glu Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile  
 225 230 235 240  
 Gly Asn Glu Phe Arg Asp Ile Pro Thr Met Ile Pro Ser Glu Ser Ala  
 245 250 255  
 Leu Ala Gly Lys Gly Asn Tyr Pro Ala Ile Val Thr Leu Asp Val Phe  
 260 265 270  
 Tyr Phe Gly Ile Glu Leu Gly Gly Arg Phe Asn Phe Gln Leu  
 275 280 285

<210> 16  
 <211> 278  
 <212> PRT  
 <213> Ehrlichia chaffeensis

<400> 16  
 Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Ala Leu Val Ser Leu Met  
 1 5 10 15  
 Ser Phe Leu Pro Gly Ile Ser Phe Ser Asp Pro Val Gln Gly Asp Asn  
 20 25 30  
 Ile Ser Gly Asn Phe Tyr Val Ser Gly Lys Tyr Met Pro Ser Ala Ser  
 35 40 45  
 His Phe Gly Met Phe Ser Ala Lys Glu Glu Lys Asn Pro Thr Val Ala  
 50 55 60

15

Leu Tyr Gly Leu Lys Gln Asp Trp Glu Gly Ile Ser Ser Ser Ser His  
 65 70 75 80  
 Asn Asp Asn His Phe Asn Asn Lys Gly Tyr Ser Phe Lys Tyr Glu Asn  
 85 90 95  
 Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Gly Gly  
 100 105 110  
 Pro Arg Val Glu Phe Glu Val Ser Tyr Glu Thr Phe Asp Val Lys Asn  
 115 120 125  
 Gln Gly Asn Asn Tyr Lys Asn Asp Ala His Arg Tyr Cys Ala Leu Gly  
 130 135 140  
 Gln Gln Asp Asn Ser Gly Ile Pro Lys Thr Ser Lys Tyr Val Leu Leu  
 145 150 155 160  
 Lys Ser Glu Gly Leu Leu Asp Ile Ser Phe Met Leu Asn Ala Cys Tyr  
 165 170 175  
 Asp Ile Ile Asn Glu Ser Ile Pro Leu Ser Pro Tyr Ile Cys Ala Gly  
 180 185 190  
 Val Gly Thr Asp Leu Ile Ser Met Phe Glu Ala Thr Asn Pro Lys Ile  
 195 200 205  
 Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Asn Pro Glu Ala  
 210 215 220  
 Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn Glu Phe  
 225 230 235 240  
 Arg Asp Ile Pro Thr Leu Lys Ala Phe Val Thr Ser Ser Ala Thr Pro  
 245 250 255  
 Asp Leu Ala Ile Val Thr Leu Ser Val Cys His Phe Gly Ile Glu Leu  
 260 265 270  
 Gly Gly Arg Phe Asn Phe  
 275

<210> 17  
 <211> 280  
 <212> PRT  
 <213> Ehrlichia chaffeensis

<400> 17  
 Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Thr Leu Val Ser Leu Met  
 1 5 10 15  
 Ser Phe Leu Pro Gly Ile Ser Phe Ser Asp Ala Val Gln Asn Asp Asn  
 20 25 30

16

Val Gly Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Val Pro Ser Val Ser  
           35                                  40                                  45  
 His Phe Gly Val Phe Ser Ala Lys Gln Glu Arg Asn Thr Thr Ile Gly  
           50                                  55                                  60  
 Val Phe Gly Leu Lys Gln Asp Trp Asp Gly Ser Thr Ile Ser Lys Asn  
           65                                  70                                  75                                  80  
 Ser Pro Glu Asn Thr Phe Asn Val Pro Asn Tyr Ser Phe Lys Tyr Glu  
                                   85                                  90                                  95  
 Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Val Gly Tyr Leu Met Asn  
                                   100                                  105                                  110  
 Gly Pro Arg Ile Glu Leu Glu Met Ser Tyr Glu Thr Phe Asp Val Lys  
                                   115                                  120                                  125  
 Asn Gln Gly Asn Asn Tyr Lys Asn Asp Ala His Lys Tyr Tyr Ala Leu  
                                   130                                  135                                  140  
 Thr His Asn Ser Gly Gly Lys Leu Ser Asn Ala Gly Asp Lys Phe Val  
                                   145                                  150                                  155                                  160  
 Phe Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Leu Met Leu Asn Ala  
                                   165                                  170                                  175  
 Cys Tyr Asp Val Ile Ser Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys  
                                   180                                  185                                  190  
 Ala Gly Val Gly Thr Asp Leu Ile Ser Met Phe Glu Ala Ile Asn Pro  
                                   195                                  200                                  205  
 Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro  
                                   210                                  215                                  220  
 Glu Ala Ser Val Phe Val Gly Gly His Phe His Lys Val Ile Gly Asn  
                                   225                                  230                                  235                                  240  
 Glu Phe Arg Asp Ile Pro Ala Met Ile Pro Ser Thr Ser Thr Leu Thr  
                                   245                                  250                                  255  
 Gly Asn His Phe Thr Ile Val Thr Leu Ser Val Cys His Phe Gly Val  
                                   260                                  265                                  270  
 Glu Leu Gly Gly Arg Phe Asn Phe  
                                   275                                  280

&lt;210&gt; 18

&lt;211&gt; 276

&lt;212&gt; PRT

&lt;213&gt; Ehrlichia chaffeensis

&lt;400&gt; 18

17

Met Asn Tyr Lys Lys Val Phe Ile Thr Ser Ala Leu Ile Ser Leu Ile  
 1 5 10 15  
 Ser Ser Leu Pro Gly Val Ser Phe Ser Asp Pro Ala Gly Ser Gly Ile  
 20 25 30  
 Asn Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Ala Ser His  
 35 40 45  
 Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly Val  
 50 55 60  
 Phe Gly Leu Lys Gln Asn Trp Asp Gly Ser Ala Ile Ser Asn Ser Ser  
 65 70 75 80  
 Pro Asn Asp Val Phe Thr Val Ser Asn Tyr Ser Phe Lys Tyr Glu Asn  
 85 90 95  
 Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp Gly  
 100 105 110  
 Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Thr Phe Asp Val Lys Asn  
 115 120 125  
 Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Cys Ala Leu Ser  
 130 135 140  
 His Asn Ser Ala Ala Asp Met Ser Ser Ala Ser Asn Asn Phe Val Phe  
 145 150 155 160  
 Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Phe Met Leu Asn Ala Cys  
 165 170 175  
 Tyr Asp Val Val Gly Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys Ala  
 180 185 190  
 Gly Ile Gly Thr Asp Leu Val Ser Met Phe Glu Ala Thr Asn Pro Lys  
 195 200 205  
 Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro Glu  
 210 215 220  
 Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn Glu  
 225 230 235 240  
 Phe Arg Asp Ile Pro Thr Ile Ile Pro Thr Gly Ser Thr Leu Ala Gly  
 245 250 255  
 Lys Gly Asn Tyr Pro Ala Ile Val Ile Leu Asp Val Cys His Phe Gly  
 260 265 270  
 Ile Glu Met Gly  
 275

<210> 19  
 <211> 287  
 <212> PRT  
 <213> Ehrlichia canis

<400> 19  
 Met Lys Tyr Lys Lys Thr Phe Thr Val Thr Ala Leu Val Leu Leu Thr  
     1                    5                    10                    15  
 Ser Phe Thr His Phe Ile Pro Phe Tyr Ser Pro Ala Arg Ala Ser Thr  
             20                    25                    30  
 Ile His Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Thr Ala Ser His  
             35                    40                    45  
 Phe Gly Ile Phe Ser Ala Lys Glu Glu Gln Ser Phe Thr Lys Val Leu  
             50                    55                    60  
 Val Gly Leu Asp Gln Arg Leu Ser His Asn Ile Ile Asn Asn Asn Asp  
             65                    70                    75                    80  
 Thr Ala Lys Ser Leu Lys Val Gln Asn Tyr Ser Phe Lys Tyr Lys Asn  
                     85                    90                    95  
 Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Ile Gly Asn  
                     100                    105                    110  
 Ser Arg Ile Glu Leu Glu Val Ser His Glu Ile Phe Asp Thr Lys Asn  
             115                    120                    125  
 Pro Gly Asn Asn Tyr Leu Asn Asp Ser His Lys Tyr Cys Ala Leu Ser  
             130                    135                    140  
 His Gly Ser His Ile Cys Ser Asp Gly Asn Ser Gly Asp Trp Tyr Thr  
             145                    150                    155                    160  
 Ala Lys Thr Asp Lys Phe Val Leu Leu Lys Asn Glu Gly Leu Leu Asp  
                     165                    170                    175  
 Val Ser Phe Met Leu Asn Ala Cys Tyr Asp Ile Thr Thr Glu Lys Met  
             180                    185                    190  
 Pro Phe Ser Pro Tyr Ile Cys Ala Gly Ile Gly Thr Asp Leu Ile Ser  
             195                    200                    205  
 Met Phe Glu Thr Thr Gln Asn Lys Ile Ser Tyr Gln Gly Lys Leu Gly  
             210                    215                    220  
 Leu Asn Tyr Thr Ile Asn Ser Arg Val Ser Val Phe Ala Gly Gly His  
             225                    230                    235                    240  
 Phe His Lys Val Ile Gly Asn Glu Phe Lys Gly Ile Pro Thr Leu Leu  
                     245                    250                    255

19

Pro Asp Gly Ser Asn Ile Lys Val Gln Gln Ser Ala Thr Val Thr Leu  
                   260                  265                  270

Asp Val Cys His Phe Gly Leu Glu Ile Gly Ser Arg Phe Phe Phe  
           275                  280                  285

&lt;210&gt; 20

&lt;211&gt; 133

&lt;212&gt; PRT

&lt;213&gt; Ehrlichia canis

&lt;400&gt; 20

Met Asn Cys Lys Lys Val Phe Thr Ile Ser Ala Leu Ile Ser Ser Ile  
   1                  5                  10                  15

Tyr Phe Leu Pro Asn Val Ser Tyr Ser Asn Pro Val Tyr Gly Asn Ser  
           20                  25                  30

Met Tyr Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Val Pro  
           35                  40                  45

His Phe Gly Ile Phe Ser Ala Glu Glu Glu Lys Lys Lys Thr Thr Val  
       50                  55                  60

Val Tyr Gly Leu Lys Glu Asn Trp Ala Gly Asp Ala Ile Ser Ser Gln  
       65                  70                  75                  80

Ser Pro Asp Asp Asn Phe Thr Ile Arg Asn Tyr Ser Phe Lys Tyr Ala  
                   85                  90                  95

Ser Asn Lys Phe Leu Gly Phe Ala Val Ala Ile Gly Tyr Ser Ile Gly  
           100                  105                  110

Ser Pro Arg Ile Glu Val Glu Met Ser Tyr Glu Ala Phe Asp Val Lys  
           115                  120                  125

Asn Gln Gly Asn Asn  
       130

&lt;210&gt; 21

&lt;211&gt; 686

&lt;212&gt; DNA

&lt;213&gt; Ehrlichia canis

&lt;400&gt; 21

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tattcctata ttacaaaaca aggcataat t caaacaaaac atcatgatac acctaatact 120

actataccaa atgaagacgg tattcaatct agcttttagct taatcaatca agacggtaaa 180

acagtaacca gccaaagattt cctagggaaa cacatgttag ttttgtttgg attctctgca 240

20

tgtaaaagca ttgccctgc agaattggga ttagtatctg aagcacttgc acaacttggt 300  
 aataatgcag acaaattaca agtaattttt attacaattg atccaaaaaa tgatactgta 360  
 gaaaaattaa aagaatttca tgaacatttt gattcaagaa ttcaaagtgt aacaggaaat 420  
 actgaagaca ttaatcaaat aattaaaaat tataaaatat atgttggaca agcagataaa 480  
 gatcatcaaa ttaaccattc tgcaataatg taccttattg acaaaaaagg atcatatctt 540  
 tcacacttca ttccagattt aaaatcacaa gaaaatcaag tagataagtt actatcttta 600  
 gttaagcagt atctgtaaat aaattcatgg aatacgttgg atgagtaggt tttttttagt 660  
 attttttagt ctaataacat tggcat 686

&lt;210&gt; 22

&lt;211&gt; 618

&lt;212&gt; DNA

&lt;213&gt; Ehrlichia chaffeensis

&lt;400&gt; 22

atgaaagtta tcaaatttat acttaatatc tgtttattat ttgcagcaat ttttctagga 60  
 tattctctacg taacaaaaca aggcattttt caagtaagag atcataacac tccaatata 120  
 aatatatcaa ataaagccag cattactact agtttttcgt tagtaaataca agatggaaat 180  
 acagtaaata gtcaagattt ttggggaaaa tacatgctag ttttatttgg attttcttca 240  
 tgtaaaagca tctgccctgc tgaattagga atagcatctg aagttctctc acagcttggt 300  
 aatgacacag acaagttaca agtaattttc attacaattg atccaacaaa tgatactgta 360  
 caaaaattaa aaacatttca tgaacatttt gatcctagaa ttcaaagtgt aacaggcagt 420  
 gcagaagata ttgaaaaaat aataaaaaat tacaaaatat atgttggaca agcagataaa 480  
 gataatcaaa ttgatcactc tgccataatg tacattatcg ataaaaaagg agaatacatt 540  
 tcacactttt ctccagattt aaaatcaaca gaaaatcaag tagataagtt actatctata 600  
 ataaaacaat atctctaa 618

&lt;210&gt; 23

&lt;211&gt; 205

&lt;212&gt; PRT

&lt;213&gt; Ehrlichia canis

&lt;400&gt; 23

Met Lys Ala Ile Lys Phe Ile Leu Asn Val Cys Leu Leu Phe Ala Ala  
 1 5 10 15

21

Ile Phe Leu Gly Tyr Ser Tyr Ile Thr Lys Gln Gly Ile Phe Gln Thr  
                   20                  25                  30  
 Lys His His Asp Thr Pro Asn Thr Thr Ile Pro Asn Glu Asp Gly Ile  
                   35                  40                  45  
 Gln Ser Ser Phe Ser Leu Ile Asn Gln Asp Gly Lys Thr Val Thr Ser  
                   50                  55                  60  
 Gln Asp Phe Leu Gly Lys His Met Leu Val Leu Phe Gly Phe Ser Ala  
                   65                  70                  75                  80  
 Cys Lys Ser Ile Cys Pro Ala Glu Leu Gly Leu Val Ser Glu Ala Leu  
                   85                  90                  95  
 Ala Gln Leu Gly Asn Asn Ala Asp Lys Leu Gln Val Ile Phe Ile Thr  
                   100                  105                  110  
 Ile Asp Pro Lys Asn Asp Thr Val Glu Lys Leu Lys Glu Phe His Glu  
                   115                  120                  125  
 His Phe Asp Ser Arg Ile Gln Met Leu Thr Gly Asn Thr Glu Asp Ile  
                   130                  135                  140  
 Asn Gln Ile Ile Lys Asn Tyr Lys Ile Tyr Val Gly Gln Ala Asp Lys  
                   145                  150                  155                  160  
 Asp His Gln Ile Asn His Ser Ala Ile Met Tyr Leu Ile Asp Lys Lys  
                   165                  170                  175  
 Gly Ser Tyr Leu Ser His Phe Ile Pro Asp Leu Lys Ser Gln Glu Asn  
                   180                  185                  190  
 Gln Val Asp Lys Leu Leu Ser Leu Val Lys Gln Tyr Leu  
                   195                  200                  205

<210> 24  
 <211> 205  
 <212> PRT  
 <213> Ehrlichia chaffeensis

<400> 24  
 Met Lys Val Ile Lys Phe Ile Leu Asn Ile Cys Leu Leu Phe Ala Ala  
                   1                  5                  10                  15  
 Ile Phe Leu Gly Tyr Ser Tyr Val Thr Lys Gln Gly Ile Phe Gln Val  
                   20                  25                  30  
 Arg Asp His Asn Thr Pro Asn Thr Asn Ile Ser Asn Lys Ala Ser Ile  
                   35                  40                  45  
 Thr Thr Ser Phe Ser Leu Val Asn Gln Asp Gly Asn Thr Val Asn Ser  
                   50                  55                  60

22

Gln Asp Phe Leu Gly Lys Tyr Met Leu Val Leu Phe Gly Phe Ser Ser  
65 70 75 80

Cys Lys Ser Ile Cys Pro Ala Glu Leu Gly Ile Ala Ser Glu Val Leu  
85 90 95

Ser Gln Leu Gly Asn Asp Thr Asp Lys Leu Gln Val Ile Phe Ile Thr  
100 105 110

Ile Asp Pro Thr Asn Asp Thr Val Gln Lys Leu Lys Thr Phe His Glu  
115 120 125

His Phe Asp Pro Arg Ile Gln Met Leu Thr Gly Ser Ala Glu Asp Ile  
130 135 140

Glu Lys Ile Ile Lys Asn Tyr Lys Ile Tyr Val Gly Gln Ala Asp Lys  
145 150 155 160

Asp Asn Gln Ile Asp His Ser Ala Ile Met Tyr Ile Ile Asp Lys Lys  
165 170 175

Gly Glu Tyr Ile Ser His Phe Ser Pro Asp Leu Lys Ser Thr Glu Asn  
180 185 190

Gln Val Asp Lys Leu Leu Ser Ile Ile Lys Gln Tyr Leu  
195 200 205

<210> 25  
<211> 618  
<212> DNA  
<213> Cowdria ruminantium

<220>  
<221> CDS  
<222> (1) .. (615)

<400> 25  
atg aag gct atc aag ttt ata ctt aat cta tgt tta cta ttt qca gca 48  
Met Lys Ala Ile Lys Phe Ile Leu Asn Leu Cys Leu Leu Phe Ala Ala  
1 5 10 15

att ttt ttg gga tat tct tac ata aca aaa caa ggt ata ttc caa cca 96  
Ile Phe Leu Gly Tyr Ser Tyr Ile Thr Lys Gln Gly Ile Phe Gln Pro  
20 25 30

aaa tta cac gac tct cct gat gtt aat ata tcg aac aaa gcg gat ata 144  
Lys Leu His Asp Ser Pro Asp Val Asn Ile Ser Asn Lys Ala Asp Ile  
35 40 45

aat act agc ttt agc tta att aat cag gat ggt att acg ata tct agt 192  
Asn Thr Ser Phe Ser Leu Ile Asn Gln Asp Gly Ile Thr Ile Ser Ser  
50 55 60

aaa gac ttc ctt gga aaa cat atg tta gtc ctt ttt ggg ttt tct tct 240

23

Lys Asp Phe Leu Gly Lys His Met Leu Val Leu Phe Gly Phe Ser Ser  
 65 70 75 80  
 tgt aaa act att tgc ccc atg gaa cta ggg tta gca tcc aca att cta 288  
 Cys Lys Thr Ile Cys Pro Met Glu Leu Gly Leu Ala Ser Thr Ile Leu  
 85 90 95  
 gat caa ctt ggc aac gaa tct gac aag tta caa gta gtc ttt ata act 336  
 Asp Gln Leu Gly Asn Glu Ser Asp Lys Leu Gln Val Val Phe Ile Thr  
 100 105 110  
 att gat cca aca aaa gat act gta gaa aca cta aaa gag ttt cac aaa 384  
 Ile Asp Pro Thr Lys Asp Thr Val Glu Thr Leu Lys Glu Phe His Lys  
 115 120 125  
 aat ttt gac tca cgg att caa atg tta aca gga aac att gaa gct att 432  
 Asn Phe Asp Ser Arg Ile Gln Met Leu Thr Gly Asn Ile Glu Ala Ile  
 130 135 140  
 aat caa ata gta caa ggg tac aaa gta tat gta ggt cag cca gac aat 480  
 Asn Gln Ile Val Gln Gly Tyr Lys Val Tyr Val Gly Gln Pro Asp Asn  
 145 150 155 160  
 gat aac caa att aac cat tct gga ata atg tat att gta gac aag aaa 528  
 Asp Asn Gln Ile Asn His Ser Gly Ile Met Tyr Ile Val Asp Lys Lys  
 165 170 175  
 gga gaa tat tta aca cat ttt gta cca gat tta aag tca aaa gag cct 576  
 Gly Glu Tyr Leu Thr His Phe Val Pro Asp Leu Lys Ser Lys Glu Pro  
 180 185 190  
 caa gtg gat aaa tta ctt tct tta att aag cag tat ctt taa 618  
 Gln Val Asp Lys Leu Leu Ser Leu Ile Lys Gln Tyr Leu  
 195 200 205

&lt;210&gt; 26

&lt;211&gt; 205

&lt;212&gt; PRT

&lt;213&gt; Cowdria ruminantium

&lt;400&gt; 26

Met Lys Ala Ile Lys Phe Ile Leu Asn Leu Cys Leu Leu Phe Ala Ala  
 1 5 10 15  
 Ile Phe Leu Gly Tyr Ser Tyr Ile Thr Lys Gln Gly Ile Phe Gln Pro  
 20 25 30  
 Lys Leu His Asp Ser Pro Asp Val Asn Ile Ser Asn Lys Ala Asp Ile  
 35 40 45  
 Asn Thr Ser Phe Ser Leu Ile Asn Gln Asp Gly Ile Thr Ile Ser Ser  
 50 55 60

24

Lys Asp Phe Leu Gly Lys His Met Leu Val Leu Phe Gly Phe Ser Ser  
 65 70 75 80  
 Cys Lys Thr Ile Cys Pro Met Glu Leu Gly Leu Ala Ser Thr Ile Leu  
 85 90 95  
 Asp Gln Leu Gly Asn Glu Ser Asp Lys Leu Gln Val Val Phe Ile Thr  
 100 105 110  
 Ile Asp Pro Thr Lys Asp Thr Val Glu Thr Leu Lys Glu Phe His Lys  
 115 120 125  
 Asn Phe Asp Ser Arg Ile Gln Met Leu Thr Gly Asn Ile Glu Ala Ile  
 130 135 140  
 Asn Gln Ile Val Gln Gly Tyr Lys Val Tyr Val Gly Gln Pro Asp Asn  
 145 150 155 160  
 Asp Asn Gln Ile Asn His Ser Gly Ile Met Tyr Ile Val Asp Lys Lys  
 165 170 175  
 Gly Glu Tyr Leu Thr His Phe Val Pro Asp Leu Lys Ser Lys Glu Pro  
 180 185 190  
 Gln Val Asp Lys Leu Leu Ser Leu Ile Lys Gln Tyr Leu  
 195 200 205

&lt;210&gt; 27

&lt;211&gt; 981

&lt;212&gt; DNA

&lt;213&gt; Cowdria ruminantium

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1) .. (978)

&lt;400&gt; 27

atg aag aaa ata ttg gtt acg ttt tta gtt gtt gtt aat gtg ttt tgt 48  
 Met Lys Lys Ile Leu Val Thr Phe Leu Val Val Val Asn Val Phe Cys  
 1 5 10 15  
 aat gct gcc att gct tca act gac tca tca gaa gat aaa cag tat att 96  
 Asn Ala Ala Ile Ala Ser Thr Asp Ser Ser Glu Asp Lys Gln Tyr Ile  
 20 25 30  
 tta att ggt act ggt tct atg act gga gta tat tat cct ata gga ggt 144  
 Leu Ile Gly Thr Gly Ser Met Thr Gly Val Tyr Tyr Pro Ile Gly Gly  
 35 40 45  
 agc ata tgt agg ttt att gca tct gat tat ggt aat gat aat aac agc 192  
 Ser Ile Cys Arg Phe Ile Ala Ser Asp Tyr Gly Asn Asp Asn Asn Ser  
 50 55 60

25

ata gtt tgt tct ata tct tct aca act ggt agc gta tat aat ctt aat	240
Ile Val Cys Ser Ile Ser Ser Thr Thr Gly Ser Val Tyr Asn Leu Asn	
65 70 75 80	
tct atg cgt tat gca aat atg gat ata ggt att att caa tct gat tta	288
Ser Met Arg Tyr Ala Asn Met Asp Ile Gly Ile Ile Gln Ser Asp Leu	
85 90 95	
gag tac tat gca tat aat ggt att ggt tta tat gaa aaa atg cca gca	336
Glu Tyr Tyr Ala Tyr Asn Gly Ile Gly Leu Tyr Glu Lys Met Pro Ala	
100 105 110	
atg agg cat cta aga ata tta tct tca tta cat aaa gaa tat ctt aca	384
Met Arg His Leu Arg Ile Leu Ser Ser Leu His Lys Glu Tyr Leu Thr	
115 120 125	
att gtt gtt agg gcg aat tct aat ata tca gtt att gat gat ata aaa	432
Ile Val Val Arg Ala Asn Ser Asn Ile Ser Val Ile Asp Asp Ile Lys	
130 135 140	
ggc aaa aga gtt aat att ggt agt cct ggt act ggt gta aga ata gca	480
Gly Lys Arg Val Asn Ile Gly Ser Pro Gly Thr Gly Val Arg Ile Ala	
145 150 155 160	
atg tta aaa ttg tta aat gaa aaa gga tgg gga aga aaa gat ttt gct	528
Met Leu Lys Leu Leu Asn Glu Lys Gly Trp Gly Arg Lys Asp Phe Ala	
165 170 175	
gtt atg gca gaa tta aaa tca tca gag caa gct caa gca tta tgt gat	576
Val Met Ala Glu Leu Lys Ser Ser Glu Gln Ala Gln Ala Leu Cys Asp	
180 185 190	
aat aaa att gat gtg atg gta gat gtt gtt gga cat cct aat gct gca	624
Asn Lys Ile Asp Val Met Val Asp Val Val Gly His Pro Asn Ala Ala	
195 200 205	
att caa gaa gca gca gca act tgt gat ata aaa ttt att tct tta gat	672
Ile Gln Glu Ala Ala Ala Thr Cys Asp Ile Lys Phe Ile Ser Leu Asp	
210 215 220	
gat gat ctc ata gat aaa tta cat act aag tat ccc tat tat aaa agg	720
Asp Asp Leu Ile Asp Lys Leu His Thr Lys Tyr Pro Tyr Tyr Lys Arg	
225 230 235 240	
gat att att agt ggt gcg tta tac agt aac tta cct gat ata caa act	768
Asp Ile Ile Ser Gly Ala Leu Tyr Ser Asn Leu Pro Asp Ile Gln Thr	
245 250 255	
gtt tca gta aaa gct tct tta ata aca act act gaa tta agc aat gag	816
Val Ser Val Lys Ala Ser Leu Ile Thr Thr Thr Glu Leu Ser Asn Glu	
260 265 270	
ttg gcc tat aaa gtt gtt aaa tct ttg gtt agc cat tta cat gaa cta	864
Leu Ala Tyr Lys Val Val Lys Ser Leu Val Ser His Leu His Glu Leu	
275 280 285	

26

cat gga att act gga gct ctt aga aat ctt act gta aaa gac atg gta 912  
 His Gly Ile Thr Gly Ala Leu Arg Asn Leu Thr Val Lys Asp Met Val  
 290 295 300

cag tca gat att aca cct tta cat gac ggt gca aaa cgt tat tat aag 960  
 Gln Ser Asp Ile Thr Pro Leu His Asp Gly Ala Lys Arg Tyr Tyr Lys  
 305 310 315 320

gaa att gga gtt ata aaa taa 981  
 Glu Ile Gly Val Ile Lys  
 325

&lt;210&gt; 28

&lt;211&gt; 326

&lt;212&gt; PRT

<213> *Cowdria ruminantium*

&lt;400&gt; 28

Met Lys Lys Ile Leu Val Thr Phe Leu Val Val Val Asn Val Phe Cys  
 1 5 10 15

Asn Ala Ala Ile Ala Ser Thr Asp Ser Ser Glu Asp Lys Gln Tyr Ile  
 20 25 30

Leu Ile Gly Thr Gly Ser Met Thr Gly Val Tyr Tyr Pro Ile Gly Gly  
 35 40 45

Ser Ile Cys Arg Phe Ile Ala Ser Asp Tyr Gly Asn Asp Asn Asn Ser  
 50 55 60

Ile Val Cys Ser Ile Ser Ser Thr Thr Gly Ser Val Tyr Asn Leu Asn  
 65 70 75 80

Ser Met Arg Tyr Ala Asn Met Asp Ile Gly Ile Ile Gln Ser Asp Leu  
 85 90 95

Glu Tyr Tyr Ala Tyr Asn Gly Ile Gly Leu Tyr Glu Lys Met Pro Ala  
 100 105 110

Met Arg His Leu Arg Ile Leu Ser Ser Leu His Lys Glu Tyr Leu Thr  
 115 120 125

Ile Val Val Arg Ala Asn Ser Asn Ile Ser Val Ile Asp Asp Ile Lys  
 130 135 140

Gly Lys Arg Val Asn Ile Gly Ser Pro Gly Thr Gly Val Arg Ile Ala  
 145 150 155 160

Met Leu Lys Leu Leu Asn Glu Lys Gly Trp Gly Arg Lys Asp Phe Ala  
 165 170 175

Val Met Ala Glu Leu Lys Ser Ser Glu Gln Ala Gln Ala Leu Cys Asp  
 180 185 190

27

Asn Lys Ile Asp Val Met Val Asp Val Val Gly His Pro Asn Ala Ala  
 195 200 205  
 Ile Gln Glu Ala Ala Ala Thr Cys Asp Ile Lys Phe Ile Ser Leu Asp  
 210 215 220  
 Asp Asp Leu Ile Asp Lys Leu His Thr Lys Tyr Pro Tyr Tyr Lys Arg  
 225 230 235 240  
 Asp Ile Ile Ser Gly Ala Leu Tyr Ser Asn Leu Pro Asp Ile Gln Thr  
 245 250 255  
 Val Ser Val Lys Ala Ser Leu Ile Thr Thr Thr Glu Leu Ser Asn Glu  
 260 265 270  
 Leu Ala Tyr Lys Val Val Lys Ser Leu Val Ser His Leu His Glu Leu  
 275 280 285  
 His Gly Ile Thr Gly Ala Leu Arg Asn Leu Thr Val Lys Asp Met Val  
 290 295 300  
 Gln Ser Asp Ile Thr Pro Leu His Asp Gly Ala Lys Arg Tyr Tyr Lys  
 305 310 315 320  
 Glu Ile Gly Val Ile Lys  
 325

&lt;210&gt; 29

&lt;211&gt; 519

&lt;212&gt; DNA

&lt;213&gt; Cowdria ruminantium

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(516)

&lt;400&gt; 29

atg aat ata ttc aat tat atg cag ata atg cct aat ata agt gtt gat 48  
 Met Asn Ile Phe Asn Tyr Met Gln Ile Met Pro Asn Ile Ser Val Asp  
 1 5 10 15  
 gca ttt gtt gca cct act gct gta att ata ggt gat gtt tgt gta aat 96  
 Ala Phe Val Ala Pro Thr Ala Val Ile Ile Gly Asp Val Cys Val Asn  
 20 25 30  
 gac aag tgt agc att tgg tat aac tca gta tta cgt gga gat gta ggc 144  
 Asp Lys Cys Ser Ile Trp Tyr Asn Ser Val Leu Arg Gly Asp Val Gly  
 35 40 45  
 caa att gtt att ggt gta ggt act aat att caa gat ggg aca ata ata 192  
 Gln Ile Val Ile Gly Val Gly Thr Asn Ile Gln Asp Gly Thr Ile Ile  
 50 55 60

28

cat gtt gat agg aaa tat ggt aat acg aat att ggc aaa aag gtt act 240  
 His Val Asp Arg Lys Tyr Gly Asn Thr Asn Ile Gly Lys Lys Val Thr  
 65 70 75 80

att ggg cat ggg tgt ata tta cat gct tgt gag ata caa gat tat gtg 288  
 Ile Gly His Gly Cys Ile Leu His Ala Cys Glu Ile Gln Asp Tyr Val  
 85 90 95

ctt gtt gga atg gga tct att att atg gat aac gtt gtg gtt gaa aag 336  
 Leu Val Gly Met Gly Ser Ile Ile Met Asp Asn Val Val Val Glu Lys  
 100 105 110

aat gca atg gtg gct gct gga tca tta gtg gta aga ggt aaa gtt gtg 384  
 Asn Ala Met Val Ala Ala Gly Ser Leu Val Val Arg Gly Lys Val Val  
 115 120 125

aaa act ggt gaa tta tgg gct ggt agg cct gca caa ttt tta aga atg 432  
 Lys Thr Gly Glu Leu Trp Ala Gly Arg Pro Ala Gln Phe Leu Arg Met  
 130 135 140

ttg tct agt gat gaa att aaa gag ata agt aaa tct gct gat aac tat 480  
 Leu Ser Ser Asp Glu Ile Lys Glu Ile Ser Lys Ser Ala Asp Asn Tyr  
 145 150 155 160

ata gag ctt gcc agt gat tac ata act ggt aag ttg taa 519  
 Ile Glu Leu Ala Ser Asp Tyr Ile Thr Gly Lys Leu  
 165 170

&lt;210&gt; 30

&lt;211&gt; 172

&lt;212&gt; PRT

&lt;213&gt; Cowdria ruminantium

&lt;400&gt; 30

Met Asn Ile Phe Asn Tyr Met Gln Ile Met Pro Asn Ile Ser Val Asp  
 1 5 10 15

Ala Phe Val Ala Pro Thr Ala Val Ile Ile Gly Asp Val Cys Val Asn  
 20 25 30

Asp Lys Cys Ser Ile Trp Tyr Asn Ser Val Leu Arg Gly Asp Val Gly  
 35 40 45

Gln Ile Val Ile Gly Val Gly Thr Asn Ile Gln Asp Gly Thr Ile Ile  
 50 55 60

His Val Asp Arg Lys Tyr Gly Asn Thr Asn Ile Gly Lys Lys Val Thr  
 65 70 75 80

Ile Gly His Gly Cys Ile Leu His Ala Cys Glu Ile Gln Asp Tyr Val  
 85 90 95

Leu Val Gly Met Gly Ser Ile Ile Met Asp Asn Val Val Val Glu Lys  
 100 105 110

29

Asn Ala Met Val Ala Ala Gly Ser Leu Val Val Arg Gly Lys Val Val  
 115 120 125

Lys Thr Gly Glu Leu Trp Ala Gly Arg Pro Ala Gln Phe Leu Arg Met  
 130 135 140

Leu Ser Ser Asp Glu Ile Lys Glu Ile Ser Lys Ser Ala Asp Asn Tyr  
 145 150 155 160

Ile Glu Leu Ala Ser Asp Tyr Ile Thr Gly Lys Leu  
 165 170

&lt;210&gt; 31

&lt;211&gt; 753

&lt;212&gt; DNA

&lt;213&gt; Cowdria ruminantium

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(750)

&lt;400&gt; 31

atg atg ata aga atc ttt ctt ttg tta ggc tta gta tta tta gta gca 48  
 Met Met Ile Arg Ile Phe Leu Leu Leu Gly Leu Val Leu Leu Val Ala  
 1 5 10 15

agt ttt cca cta tta aat aac tgg cta tct aat cat tct ggt aag tct 96  
 Ser Phe Pro Leu Leu Asn Asn Trp Leu Ser Asn His Ser Gly Lys Ser  
 20 25 30

act aca ttg gat aag gat gca gtt ata tct ata gtt gag gaa tat ata 144  
 Thr Thr Leu Asp Lys Asp Ala Val Ile Ser Ile Val Glu Glu Tyr Ile  
 35 40 45

acc aat tat cct cag agg gta ata gat tta ctt act aca ggc caa gca 192  
 Thr Asn Tyr Pro Gln Arg Val Ile Asp Leu Leu Thr Thr Gly Gln Ala  
 50 55 60

caa gca gaa aga gca gag ctt act gaa aat att aaa aaa tat aaa tct 240  
 Gln Ala Glu Arg Ala Glu Leu Thr Glu Asn Ile Lys Lys Tyr Lys Ser  
 65 70 75 80

gag ctt gaa gat att gca tac cca tct gct ggc aat aaa gac agt aaa 288  
 Glu Leu Glu Asp Ile Ala Tyr Pro Ser Ala Gly Asn Lys Asp Ser Lys  
 85 90 95

att gca ttt att gag ttc ttc gat tac tct tgt ggt tat tgt aaa atg 336  
 Ile Ala Phe Ile Glu Phe Phe Asp Tyr Ser Cys Gly Tyr Cys Lys Met  
 100 105 110

atg ttt gaa gat atc aaa caa att ata aaa gat ggt aag gta cgt gtt 384  
 Met Phe Glu Asp Ile Lys Gln Ile Ile Lys Asp Gly Lys Val Arg Val  
 115 120 125

30

att ttt aga gat ttt cca ata ctt ggg gaa tgg tgg tta aag gct gtt 432  
 Ile Phe Arg Asp Phe Pro Ile Leu Gly Glu Ser Ser Leu Lys Ala Val  
 130 135 140

aaa gca gca ttg gct gta cat ctt atc aat cca agt aaa tac ttg gac 480  
 Lys Ala Ala Leu Ala Val His Leu Ile Asn Pro Ser Lys Tyr Leu Asp  
 145 150 155 160

ttc tat tat gca gca tta aat cat aaa cag cca ttt aat gat gaa tct 528  
 Phe Tyr Tyr Ala Ala Leu Asn His Lys Gln Pro Phe Asn Asp Glu Ser  
 165 170 175

ata ctt aat ata gtt aaa tca ctt gaa att tca gaa gag gaa ttt aaa 576  
 Ile Leu Asn Ile Val Lys Ser Leu Glu Ile Ser Glu Glu Glu Phe Lys  
 180 185 190

gat tct tta tct aaa aat tct agt act att gat aag atg ata gag tcc 624  
 Asp Ser Leu Ser Lys Asn Ser Ser Thr Ile Asp Lys Met Ile Glu Ser  
 195 200 205

act aga aat ctg gct gag aag tta aat atc aga ggt act cct gct ctt 672  
 Thr Arg Asn Leu Ala Glu Lys Leu Asn Ile Arg Gly Thr Pro Ala Leu  
 210 215 220

ata ata ggt gat gca ttc att ggg gga gct gca gat tta tca act tta 720  
 Ile Ile Gly Asp Ala Phe Ile Gly Gly Ala Ala Asp Leu Ser Thr Leu  
 225 230 235 240

aga agt aaa ata gta gaa cag cag gaa caa taa 753  
 Arg Ser Lys Ile Val Glu Gln Gln Glu Gln  
 245 250

<210> 32  
 <211> 250  
 <212> PRT  
 <213> Cowdria ruminantium

<400> 32  
 Met Met Ile Arg Ile Phe Leu Leu Leu Gly Leu Val Leu Leu Val Ala  
 1 5 10 15

Ser Phe Pro Leu Leu Asn Asn Trp Leu Ser Asn His Ser Gly Lys Ser  
 20 25 30

Thr Thr Leu Asp Lys Asp Ala Val Ile Ser Ile Val Glu Glu Tyr Ile  
 35 40 45

Thr Asn Tyr Pro Gln Arg Val Ile Asp Leu Leu Thr Thr Gly Gln Ala  
 50 55 60

Gln Ala Glu Arg Ala Glu Leu Thr Glu Asn Ile Lys Lys Tyr Lys Ser  
 65 70 75 80

31

Glu Leu Glu Asp Ile Ala Tyr Pro Ser Ala Gly Asn Lys Asp Ser Lys  
85 90 95

Ile Ala Phe Ile Glu Phe Phe Asp Tyr Ser Cys Gly Tyr Cys Lys Met  
100 105 110

Met Phe Glu Asp Ile Lys Gln Ile Ile Lys Asp Gly Lys Val Arg Val  
115 120 125

Ile Phe Arg Asp Phe Pro Ile Leu Gly Glu Ser Ser Leu Lys Ala Val  
130 135 140

Lys Ala Ala Leu Ala Val His Leu Ile Asn Pro Ser Lys Tyr Leu Asp  
145 150 155 160

Phe Tyr Tyr Ala Ala Leu Asn His Lys Gln Pro Phe Asn Asp Glu Ser  
165 170 175

Ile Leu Asn Ile Val Lys Ser Leu Glu Ile Ser Glu Glu Glu Phe Lys  
180 185 190

Asp Ser Leu Ser Lys Asn Ser Ser Thr Ile Asp Lys Met Ile Glu Ser  
195 200 205

Thr Arg Asn Leu Ala Glu Lys Leu Asn Ile Arg Gly Thr Pro Ala Leu  
210 215 220

Ile Ile Gly Asp Ala Phe Ile Gly Gly Ala Ala Asp Leu Ser Thr Leu  
225 230 235 240

Arg Ser Lys Ile Val Glu Gln Gln Glu Gln  
245 250

&lt;210&gt; 33

&lt;211&gt; 450

&lt;212&gt; DNA

&lt;213&gt; Cowdria ruminantium

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1) .. (447)

&lt;400&gt; 33

atg cat aga tca aat att att gaa att ttt ata gga ttc cta gtg tta 48  
Met His Arg Ser Asn Ile Ile Glu Ile Phe Ile Gly Phe Leu Val Leu  
1 5 10 15

gca gga gca ata tct att ggg ata ata gca ttt aac aaa tta cca tat 96  
Ala Gly Ala Ile Ser Ile Gly Ile Ile Ala Phe Asn Lys Leu Pro Tyr  
20 25 30

aaa aat acc ttg cgt aat tgt tat aca gtt aaa gca ttt ttc tca aat 144  
Lys Asn Thr Leu Arg Asn Cys Tyr Thr Val Lys Ala Phe Phe Ser Asn  
35 40 45

32

gta gat ggg ttg gac ata gga gat gaa gta aca ata tca gga gta aaa 192  
 Val Asp Gly Leu Asp Ile Gly Asp Glu Val Thr Ile Ser Gly Val Lys  
 50 55 60

ata ggt aca gta act tca ata tca ttg aat gaa agc tat act cct ata 240  
 Ile Gly Thr Val Thr Ser Ile Ser Leu Asn Glu Ser Tyr Thr Pro Ile  
 65 70 75 80

gta aca atg tgc ata cag aaa aat atc tta cta cct tca gat agt tca 288  
 Val Thr Met Cys Ile Gln Lys Asn Ile Leu Leu Pro Ser Asp Ser Ser  
 85 90 95

gca tct ata tta aac agc aat atg tta gga aaa aag cac att gat atc 336  
 Ala Ser Ile Leu Asn Ser Asn Met Leu Gly Lys Lys His Ile Asp Ile  
 100 105 110

gaa ctt gga tca gat caa gaa gtc atc gta agt gaa ggt tta ata gaa 384  
 Glu Leu Gly Ser Asp Gln Glu Val Ile Val Ser Glu Gly Leu Ile Glu  
 115 120 125

cat aca cat tca gat tta agt ttc aat gca att att gct aaa ata ata 432  
 His Thr His Ser Asp Leu Ser Phe Asn Ala Ile Ile Ala Lys Ile Ile  
 130 135 140

gat tca ctt att aag tag 450  
 Asp Ser Leu Ile Lys  
 145

&lt;210&gt; 34

&lt;211&gt; 149

&lt;212&gt; PRT

&lt;213&gt; Cowdria ruminantium

&lt;400&gt; 34

Met His Arg Ser Asn Ile Ile Glu Ile Phe Ile Gly Phe Leu Val Leu  
 1 5 10 15

Ala Gly Ala Ile Ser Ile Gly Ile Ile Ala Phe Asn Lys Leu Pro Tyr  
 20 25 30

Lys Asn Thr Leu Arg Asn Cys Tyr Thr Val Lys Ala Phe Phe Ser Asn  
 35 40 45

Val Asp Gly Leu Asp Ile Gly Asp Glu Val Thr Ile Ser Gly Val Lys  
 50 55 60

Ile Gly Thr Val Thr Ser Ile Ser Leu Asn Glu Ser Tyr Thr Pro Ile  
 65 70 75 80

Val Thr Met Cys Ile Gln Lys Asn Ile Leu Leu Pro Ser Asp Ser Ser  
 85 90 95

Ala Ser Ile Leu Asn Ser Asn Met Leu Gly Lys Lys His Ile Asp Ile  
 100 105 110

33

Glu Leu Gly Ser Asp Gln Glu Val Ile Val Ser Glu Gly Leu Ile Glu  
115 120 125

His Thr His Ser Asp Leu Ser Phe Asn Ala Ile Ile Ala Lys Ile Ile  
130 135 140

Asp Ser Leu Ile Lys  
145



(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
2 November 2000 (02.11.2000)

PCT

(10) International Publication Number  
**WO 00/65063 A3**

(51) International Patent Classification<sup>7</sup>: C12N 15/31,  
C07K 14/29, A61K 39/02, C12Q 1/68

(21) International Application Number: PCT/US00/10886

(22) International Filing Date: 21 April 2000 (21.04.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/130,725 22 April 1999 (22.04.1999) US

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(81) Designated States (*national*): AU, TZ, ZA.

(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), European patent  
(AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,  
MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM,  
GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— With international search report.

(88) Date of publication of the international search report:  
12 April 2001

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: NUCLEIC ACID VACCINES AGAINST RICKETTSIAL DISEASES AND METHODS OF USE

(57) Abstract: Described are nucleic acid vaccines containing genes to protect animals or humans against rickettsial diseases. Also described are polypeptides and methods of using these polypeptides to detect antibodies to pathogens.



WO 00/65063 A3

## INTERNATIONAL SEARCH REPORT

Internat'l Application No

PCT/US 00/10886

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/31 C07K14/29 A61K39/02 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 16554 A (UNIV FLORIDA) 23 April 1998 (1998-04-23) the whole document	1-24
X	--- BOWIE MICHAEL V ET AL: "Potential value of major antigenic protein 2 for serological diagnosis of heartwater and related Ehrlichial infections." CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, vol. 6, no. 2, March 1999 (1999-03), pages 209-215, XP000939015 ISSN: 1071-412X the whole document --- -/-	1-4, 7-13, 21-24

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

5 September 2000

Date of mailing of the international search report

22. 12. 2000

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## INTERNATIONAL SEARCH REPORT

Internat'l Application No

PCT/US 00/10886

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NYIKA A ET AL.: "A DNA vaccine protects mice against the rickettsial agent Cowdria ruminantium." PARASITE IMMUNOLOGY (OXFORD), vol. 20, no. 3, March 1998 (1998-03), pages 111-119, XP000939081 ISSN: 0141-9838 the whole document ---	1-4, 6-14, 17-19
X	MAHAN S M ET AL: "Molecular cloning of a gene encoding the immunogenic 21 kDa protein of Cowdria ruminantium." MICROBIOLOGY (READING), vol. 140, no. 8, 1994, pages 2135-2142, XP000939016 the whole document -----	1-4, 7-13, 21-24

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/10886

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
**see FURTHER INFORMATION sheet PCT/ISA/210**
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

**see additional sheet**

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**1-24**

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 10 to 19 are directed to a method of treatment of the human/animal body, and claim 20 (as far as an in vivo method is concerned) is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-24

1.1. Claims: 1-2,6-7,10-11,17-19,21-22 (all partially)

A composition comprising a polynucleotide encoding an antigen from *Rickettsia* spp. and methods for using it in protection of a host against a disease or death, or in diagnostic.

1.2. Claims: 1-4,6-13,17-24 (all partially), and claims 5, 15 (totally)

Compositions comprising SEQ IDs 3,4; 7,14; 8,15; 9,16; 10,17; 11,18 and 22,24 (corresponding to the MAP1, VSA1 to VSA5 and MAP2 antigens from *Ehrlichia chaffeensis*) and methods for using them in protection of a host against a disease or death, or in diagnostic.

1.3. Claims: 1-4,6-13,17-24 (all partially)

Compositions comprising SEQ IDs 12,19; 13,20 and 21,23 (corresponding to the VSA1, VSA2 and MAP2 antigens from *Ehrlichia canis*) and methods for using them in protection of a host against a disease or death, or in diagnostic.

1.4. Claims: 1-4,6-13,17-19, 21-24 (all partially) and claim 16 (totally)

A compositions comprising SEQ IDs 4 and 5 (corresponding to the MSP-4 antigen from *Anaplasma marginale*) and methods for using it in protection of a host against a disease or death, or in diagnostic.

1.5. Claims: 1-4,6-13,17-19, 21-24 (all partially) and claim 14 (totally)

Compositions comprising SEQ IDs 1,2 and 25,26 (corresponding to the antigens MAP1 and MAP2 from *Cowdria ruminantium*) and methods for using them in protection of a host against a disease or death, or in diagnostic.

2. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 27 and 28 (corresponding to the 1hworf3 antigen from *Cowdria ruminantium*) and methods

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

for using it in protection of a host against a disease or death, or in diagnostic.

3. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 29 and 30 (corresponding to the 4hworf1 antigen from Cowdria ruminantium) and methods for using it in protection of a host against a disease or death, or in diagnostic.

4. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 31 and 32 (corresponding to the 18hworf1 antigen from Cowdria ruminantium) and methods for using it in protection of a host against a disease or death, or in diagnostic.

5. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 33 and 34 (corresponding to the 3gdorf3 antigen from Cowdria ruminantium) and methods for using it in protection of a host against a disease or death, or in diagnostic.

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/10886

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9816554 A	23-04-1998	US 6025338 A AU 4913097 A ZA 9709320 A	15-02-2000 11-05-1998 16-03-1999
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CORRECTED VERSION

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
2 November 2000 (02.11.2000)

PCT

(10) International Publication Number  
**WO 00/65063 A3**

(51) International Patent Classification<sup>7</sup>: **C12N 15/31**,  
C07K 14/29, A61K 39/02, C12Q 1/68

1079 Outlaw Trail, Hamilton, MT 59840 (US). **ALLEMAN, Arthur, R.**: 13337 S.W. 39th Avenue, Alachua, FL 32615 (US).

(21) International Application Number: PCT/US00/10886

(22) International Filing Date: 21 April 2000 (21.04.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/130,725 22 April 1999 (22.04.1999) US

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(81) Designated States (*national*): AU, TZ, ZA.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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Published:

— with international search report

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(88) Date of publication of the international search report:  
12 April 2001

(48) Date of publication of this corrected version:  
14 February 2002

(15) Information about Correction:  
see PCT Gazette No. 07/2002 of 14 February 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NUCLEIC ACID VACCINES AGAINST RICKETTSIAL DISEASES AND METHODS OF USE

(57) Abstract: Described are nucleic acid vaccines containing genes to protect animals or humans against rickettsial diseases. Also described are polypeptides and methods of using these polypeptides to detect antibodies to pathogens.

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## DESCRIPTION

### NUCLEIC ACID VACCINES AGAINST RICKETTSIAL DISEASES AND METHODS OF USE

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This invention was made with government support under USAID Grant No. LAG-1328-G-00-3030-00. The government has certain rights in this invention.

#### Technical Field

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This invention relates to nucleic acid vaccines for rickettsial diseases of animals, including humans.

#### Background of the Invention

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The rickettsias are a group of small bacteria commonly transmitted by arthropod vectors to man and animals, in which they may cause serious disease. The pathogens causing human rickettsial diseases include the agent of epidemic typhus, *Rickettsia prowazekii*, which has resulted in the deaths of millions of people during wartime and natural disasters. The causative agents of spotted fever, e.g., *Rickettsia rickettsii* and *Rickettsia conorii*, are also included within this group. Recently, new types of human rickettsial disease caused by members of the tribe *Ehrlichiae* have been described. *Ehrlichiae* infect leukocytes and endothelial cells of many different mammalian species, some of them causing serious human and veterinary diseases. Over 400 cases of human ehrlichiosis, including some fatalities, caused by *Ehrlichia chaffeensis* have now been reported. Clinical signs of human ehrlichiosis are similar to those of Rocky Mountain spotted fever, including fever, nausea, vomiting, headache, and rash.

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Heartwater is another infectious disease caused by a rickettsial pathogen, namely *Cowdria ruminantium*, and is transmitted by ticks of the genus *Amblyomma*. The disease occurs throughout most of Africa and has an estimated endemic area of about 5 million square miles. In endemic areas, heartwater is a latent infection in indigenous breeds of cattle that have been subjected to centuries of natural selection. The problems occur where the disease contacts susceptible or naive cattle and other ruminants. Heartwater has been confirmed to be on the island of Guadeloupe in the Caribbean and is spreading through the Caribbean Islands. The tick vectors responsible for spreading this disease are already present on the American mainland and threaten the livestock industry in North and South America.

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In acute cases of heartwater, animals exhibit a sudden rise in temperature, signs of anorexia, cessation of rumination, and nervous symptoms including staggering, muscle twitching, and convulsions. Death usually occurs during these convulsions. Peracute cases of the disease occur where the animal collapses and dies in convulsions having shown no preliminary symptoms. Mortality is high in susceptible animals. Angora sheep infected with the disease have a 90% mortality rate while susceptible cattle strains have up to a 60% mortality rate.

If detected early, tetracycline or chloramphenicol treatment are effective against rickettsial infections, but symptoms are similar to numerous other infections and there are no satisfactory diagnostic tests (Helmick, C., K. Bernard, L. D'Angelo [1984] *J. Infect. Dis.* 150:480).

Animals which have recovered from heartwater are resistant to further homologous, and in some cases heterologous, strain challenge. It has similarly been found that persons recovering from a rickettsial infection may develop a solid and lasting immunity. Individuals recovered from natural infections are often immune to multiple isolates and even species. For example, guinea pigs immunized with a recombinant *R. conorii* protein were partially protected even against *R. rickettsii* (Vishwanath, S., G. McDonald, N. Watkins [1990] *Infect. Immun.* 58:646). It is known that there is structural variation in rickettsial antigens between different geographical isolates. Thus, a functional recombinant vaccine against multiple isolates would need to contain multiple epitopes, e.g., protective T and B cell epitopes, shared between isolates. It is believed that serum antibodies do not play a significant role in the mechanism of immunity against rickettsia (Uilenberg, G. [1983] *Advances in Vet. Sci. and Comp. Med.* 27:427-480; Du Plessis, Plessis, J.L. [1970] *Onderstepoort J. Vet. Res.* 37(3):147-150).

Vaccines based on inactivated or attenuated rickettsiae have been developed against certain rickettsial diseases, for example against *R. prowazekii* and *R. rickettsii*. However, these vaccines have major problems or disadvantages, including undesirable toxic reactions, difficulty in standardization, and expense (Woodward, T. [1981] "Rickettsial diseases: certain unsettled problems in their historical perspective," In *Rickettsia and Rickettsial Diseases*, W. Burgdorfer and R. Anacker, eds., Academic Press, New York, pp. 17-40).

A vaccine currently used in the control of heartwater is composed of live infected sheep blood. This vaccine also has several disadvantages. First, expertise is required for the intravenous inoculation techniques required to administer this vaccine. Second, vaccinated animals may experience shock and so require daily monitoring for a period after vaccination. There is a possibility of death due to shock throughout this monitoring period, and the drugs

needed to treat any shock induced by vaccination are costly. Third, blood-borne parasites may be present in the blood vaccine and be transmitted to the vaccinees. Finally, the blood vaccine requires a cold chain to preserve the vaccine.

Clearly, a safer, more effective vaccine that is easily administered would be particularly advantageous. For these reasons, and with the advent of new methods in biotechnology, investigators have concentrated recently on the development of new types of vaccines, including recombinant vaccines. However, recombinant vaccine antigens must be carefully selected and presented to the immune system such that shared epitopes are recognized. These factors have contributed to the search for effective vaccines.

A protective vaccine against rickettsiae that elicits a complete immune response can be advantageous. A few antigens which potentially can be useful as vaccines have now been identified and sequenced for various pathogenic rickettsiae. The genes encoding the antigens and that can be employed to recombinantly produce those antigens have also been identified and sequenced. Certain protective antigens identified for *R. rickettsii*, *R. conorii*, and *R. prowazekii* (e.g., rOmpA and rOmpB) are large (>100 kDa), dependent on retention of native conformation for protective efficacy, but are often degraded when produced in recombinant systems. This presents technical and quality-control problems if purified recombinant proteins are to be included in a vaccine. The mode of presentation of a recombinant antigen to the immune system can also be an important factor in the immune response.

Nucleic acid vaccination has been shown to induce protective immune responses in non-viral systems and in diverse animal species (Special Conference Issue, WHO meeting on nucleic acid vaccines [1994] *Vaccine* 12:1491). Nucleic acid vaccination has induced cytotoxic lymphocyte (CTL), T-helper 1, and antibody responses, and has been shown to be protective against disease (Ulmer, J., J. Donnelly, S. Parker *et al* [1993] *Science* 259:1745). For example, direct intramuscular injection of mice with DNA encoding the influenza nucleoprotein caused the production of high titer antibodies, nucleoprotein-specific CTLs, and protection against viral challenge. Immunization of mice with plasmid DNA encoding the *Plasmodium yoelii* circumsporozoite protein induced high antibody titers against malaria sporozoites and CTLs, and protection against challenge infection (Sedegah, M., R. Hedstrom, P. Hobart, S. Hoffman [1994] *Proc. Natl. Acad. Sci. USA* 91:9866). Cattle immunized with plasmids encoding bovine herpesvirus 1 (BHV-1) glycoprotein IV developed neutralizing antibody and were partially protected (Cox, G., T. Zamb, L. Babiuk [1993] *J. Virol.* 67:5664). However, it has been a question in the field of immunization whether the recently discovered technology of nucleic acid vaccines can provide improved protection against an antigenic drift variant. Moreover, it has

not heretofore been recognized or suggested that nucleic acid vaccines may be successful to protect against rickettsial disease or that a major surface protein conserved in rickettsia was protective against disease.

#### Brief Summary of the Invention

Disclosed and claimed here are novel vaccines for conferring immunity to rickettsia infection, including *Cowdria ruminantium* causing heartwater. Also disclosed are novel nucleic acid compositions and methods of using those compositions, including to confer immunity in a susceptible host. Also disclosed are novel materials and methods for diagnosing infections by *Ehrlichia* in humans or animals.

One aspect of the subject invention concerns a nucleic acid, e.g., DNA or mRNA, vaccine containing the major antigenic protein 1 gene (MAP1) or the major antigenic protein 2 gene (MAP2) of rickettsial pathogens. In one embodiment, the nucleic acid vaccines can be driven by the human cytomegalovirus (HCMV) enhancer-promoter. In studies immunizing mice by intramuscular injection of a DNA vaccine composition according to the subject invention, immunized mice seroconverted and reacted with MAP1 in antigen blots. Splenocytes from immunized mice, but not from control mice immunized with vector only, proliferated in response to recombinant MAP1 and rickettsial antigens in *in vitro* lymphocyte proliferation tests. In experiments testing different DNA vaccine dose regimens, increased survival rates as compared to controls were observed on challenge with rickettsia. Accordingly, the subject invention concerns the discovery that DNA vaccines can induce protective immunity against rickettsial disease or death resulting therefrom.

The subject invention further concerns the genes designated *Cowdria ruminantium map 2*, *Cowdria ruminantium 1hworf3*, *Cowdria ruminantium 4hworf1*, *Cowdria ruminantium 18hworf1*, and *Cowdria ruminantium 3gdorf3* and the use of these genes in diagnostic and therapeutic applications. The subject invention further concerns the proteins encoded by the exemplified genes, antibodies to these proteins, and the use of such antibodies and proteins in diagnostic and therapeutic applications.

In one embodiment of the subject invention, the polynucleotide vaccines are administered in conjunction with an antigen. In a preferred embodiment, the antigen is the polypeptide which is encoded by the polynucleotide administered as the polynucleotide vaccine. As a particularly preferred embodiment, the antigen is administered as a booster subsequent to the initial administration of the polynucleotide vaccine.

### Brief Description of the Drawings

**Figures 1A-1C** show a comparison of the amino acid sequences from alignment of the three rickettsial proteins, namely, *Cowdria ruminantium* (*C.r.*), *Ehrlichia chaffeensis* (*E.c.*), and *Anaplasma marginale* (*A.m.*).

**Figures 2A-2C** shows the DNA sequence of the 28 kDa gene locus cloned from *E. chaffeensis* (Fig. 2A-2B) and *E. canis* (Fig. 2C). One letter amino acid codes for the deduced protein sequences are presented below the nucleotide sequence. The proposed sigma-70-like promoter sequences (38) are presented in bold and underlined text as -10 and -35 (consensus -35 and -10 sequences are TTGACA and TATAAT, respectively). Similarly, consensus ribosomal binding sites and transcription terminator sequences (bold letter sequence) are identified. G-rich regions identified in the *E. chaffeensis* sequence are underlined. The conserved sequences from within the coding regions selected for RT-PCR assay are identified with italics and underlined text.

**Figure 3A** shows the complete sequence of the MAP2 homolog of *Ehrlichia canis*. The arrow (→) represents the predicted start of the mature protein. The asterisk (\*) represents the stop codon. Underlined nucleotides 5' to the open reading frame with -35 and -10 below represent predicted promoter sequences. Double underlined nucleotides represent the predicted ribosomal binding site. Underlined nucleotides 3' to the open reading frame represent possible transcription termination sequences.

**Figure 3B** shows the complete sequence of the MAP2 homolog of *Ehrlichia chaffeensis*. The arrow (→) represents the predicted start of the mature protein. The asterisk (\*) represents the stop codon. Underlined nucleotides 5' to the open reading frame with -35 and -10 below represent predicted promoter sequences. Double underlined nucleotides represent the predicted ribosomal binding site. Underlined nucleotides 3' to the open reading frame represent possible transcription termination sequences.

### Brief Description of the Sequences

**SEQ ID NO. 1** is the coding sequence of the MAP1 gene from *Cowdria ruminantium* (Highway isolate).

**SEQ ID NO. 2** is the polypeptide encoded by the polynucleotide of SEQ ID NO. 1.

**SEQ ID NO. 3** is the coding sequence of the MAP1 gene from *Ehrlichia chaffeensis*.

**SEQ ID NO. 4** is the polypeptide encoded by the polynucleotide of SEQ ID NO. 3.

**SEQ ID NO. 5** is the *Anaplasma marginale* MSP4 gene coding sequence.

**SEQ ID NO. 6** is the polypeptide encoded by the polynucleotide of SEQ ID NO. 5.

SEQ ID NO. 7 is a partial coding sequence of the VSA1 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

SEQ ID NO. 8 is the coding sequence of the VSA2 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

5 SEQ ID NO. 9 is the coding sequence of the VSA3 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

SEQ ID NO. 10 is the coding sequence of the VSA4 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

10 SEQ ID NO. 11 is a partial coding sequence of the VSA5 gene from *Ehrlichia chaffeensis*, also shown in Figures 2A-2B.

SEQ ID NO. 12 is the coding sequence of the VSA1 gene from *Ehrlichia canis*, also shown in Figure 2C.

SEQ ID NO. 13 is a partial coding sequence of the VSA2 gene from *Ehrlichia canis*, also shown in Figure 2C.

15 SEQ ID NO. 14 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 7, also shown in Figures 2A-2B.

SEQ ID NO. 15 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 8, also shown in Figures 2A-2B.

20 SEQ ID NO. 16 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 9, also shown in Figures 2A-2B.

SEQ ID NO. 17 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 10, also shown in Figures 2A-2B.

SEQ ID NO. 18 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 11, also shown in Figures 2A-2B.

25 SEQ ID NO. 19 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 12, also shown in Figure 2C.

SEQ ID NO. 20 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 13, also shown in Figure 2C.

30 SEQ ID NO. 21 is the coding sequence of the MAP2 gene from *Ehrlichia canis*, also shown in Figure 3A.

SEQ ID NO. 22 is the coding sequence of the MAP2 gene from *Ehrlichia chaffeensis*, also shown in Figure 3B.

SEQ ID NO. 23 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 21, also shown in Figure 3A.

SEQ ID NO. 24 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 22, also shown in Figure 3B.

SEQ ID NO. 25 is the coding sequence of the *map2* gene from *Cowdria ruminantium*.

SEQ ID NO. 26 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 25.

5 SEQ ID NO. 27 is the coding sequence of the *ihworf3* gene from *Cowdria ruminantium*.

SEQ ID NO. 28 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 27.

SEQ ID NO. 29 is the coding sequence of the *4hworf1* gene from *Cowdria ruminantium*.

SEQ ID NO. 30 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 29.

10 SEQ ID NO. 31 is the coding sequence of the *18hworf1* gene from *Cowdria ruminantium*.

SEQ ID NO. 32 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 31.

SEQ ID NO. 33 is the coding sequence of the *3gdorf3* gene from *Cowdria ruminantium*.

SEQ ID NO. 34 is the polypeptide encoded by the polynucleotide of SEQ ID NO. 33.

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#### Detailed Disclosure of the Invention

In one embodiment, the subject invention concerns a novel strategy, termed nucleic acid vaccination, for eliciting an immune response protective against rickettsial disease. The subject invention also concerns novel compositions that can be employed according to this novel strategy for eliciting a protective immune response.

20 According to the subject invention, recombinant DNA or mRNA encoding an antigen of interest is inoculated directly into the human or animal host where an immune response is induced. Prokaryotic signal sequences may be deleted from the nucleic acid encoding an antigen of interest. Advantageously, problems of protein purification, as can be encountered with antigen delivery using live vectors, can be virtually eliminated by employing the compositions or methods according to the subject invention. Unlike live vector delivery, the subject invention can provide a further advantage in that the DNA or RNA does not replicate in the host, but remains episomal. See, for example, Wolff, J.A., J.J. Ludike, G. Acsadi, P. Williams, A. Jani (1992) *Hum. Mol. Genet.* 1:363. A complete immune response can be obtained as recombinant antigen is synthesized intracellularly and presented to the host immune system in the context of autologous class I and class II MHC molecules.

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In one embodiment, the subject invention concerns nucleic acids and compositions comprising those nucleic acids that can be effective in protecting an animal from disease or death caused by rickettsia. For example, a nucleic acid vaccine of the subject invention has been

shown to be protective against *Cowdria ruminantium*, the causative agent of heartwater in domestic ruminants. Accordingly, nucleotide sequences of rickettsial genes, as described herein, can be used as nucleic acid vaccines against human and animal rickettsial diseases.

In one embodiment of the subject invention, the polynucleotide vaccines are administered in conjunction with an antigen. In a preferred embodiment, the antigen is the polypeptide which is encoded by the polynucleotide administered as the polynucleotide vaccine. As a particularly preferred embodiment, the antigen is administered as a booster subsequent to the initial administration of the polynucleotide vaccine. In another embodiment of the invention, the polynucleotide vaccine is administered in the form of a "cocktail" which contains at least two of the nucleic acid vaccines of the subject invention. The "cocktail" may be administered in conjunction with an antigen or an antigen booster as described above.

The MAP1 gene, which can be used to obtain this protection, is also present in other rickettsiae including *Anaplasma marginale*, *Ehrlichia canis*, and in a causative agent of human ehrlichiosis, *Ehrlichia chaffeensis* (van Vliet, A., F. Jongejan, M. van Kleef, B. van der Zeijst [1994] *Infect. Immun.* 62:1451). The MAP1 gene or a MAP1-like gene can also be found in certain *Rickettsia* spp. MAP1-like genes from *Ehrlichia chaffeensis* and *Ehrlichia canis* have now been cloned and sequenced. These MAP-1 homologs are also referred to herein as Variable Surface Antigen (VSA) genes.

The present invention also concerns polynucleotides encoding MAP2 or MAP2 homologs from *Ehrlichia canis* and *Ehrlichia chaffeensis*. MAP2 polynucleotide sequences of the invention can be used as vaccine compositions and in diagnostic assays. The polynucleotides can also be used to produce the MAP2 polypeptides encoded thereby.

The subject invention further concerns the genes designated *Cowdria ruminantium map 2*, *Cowdria ruminantium 1hworf3*, *Cowdria ruminantium 4hworf1*, *Cowdria ruminantium 18hworf1*, and *Cowdria ruminantium 3gdorf3* and the use of these genes in diagnostic and therapeutic applications. The subject invention further concerns the proteins encoded by the exemplified genes, antibodies to these proteins, and the use of such antibodies and proteins in diagnostic and therapeutic applications.

Compositions comprising the subject polynucleotides can include appropriate nucleic acid vaccine vectors (plasmids), which are commercially available (e.g., Vical, San Diego, CA). In addition, the compositions can include a pharmaceutically acceptable carrier, e.g., saline. The pharmaceutically acceptable carriers are well known in the art and also are commercially available. For example, such acceptable carriers are described in E.W. Martin's *Remington's Pharmaceutical Science*, Mack Publishing Company, Easton, PA.

The subject invention also concerns polypeptides encoded by the subject polynucleotides. Specifically exemplified are the polypeptides encoded by the MAP-1 and VSA genes of *C. rumimontium*, *E. chaffeensis*, *E. canis* and the MP4 gene of *Anaplasma marginale*. Polypeptides encoded by *E. chaffeensis* and *E. canis* MAP2 genes are also exemplified herein.

Also encompassed within the scope of the present invention are fragments and variants of the exemplified polynucleotides and polypeptides. Fragments would include, for example, portions of the exemplified sequences wherein procaryotic signal sequences have been removed. Examples of the removal of such sequences are given in Example 3. Variants include polynucleotides and/or polypeptides having base or amino acid additions, deletions and substitutions in the sequence of the subject molecule so long as those variants have substantially the same activity or serologic reactivity as the native molecules. Also included are allelic variants of the subject polynucleotides. The polypeptides of the present invention can be used to raise antibodies that are reactive with the polypeptides disclosed herein. The polypeptides and polynucleotides can also be used as molecular weight markers.

Another aspect of the subject invention concerns antibodies reactive with MAP-1 and MAP2 polypeptides disclosed herein. Antibodies can be monoclonal or polyclonal and can be produced using standard techniques known in the art. Antibodies of the invention can be used in diagnostic and therapeutic applications.

In a specific embodiment, the subject invention concerns a DNA vaccine (e.g., VCL1010/MAP1) containing the major antigenic protein 1 gene (MAP1) driven by the human cytomegalovirus (HCMV) enhancer-promoter. In a specific example, this vaccine was injected intramuscularly into 8-10 week-old female DBA/2 mice after treating them with 50 µl/muscle of 0.5% bupivacaine 3 days previously. Up to 75% of the VCL1010/MAP1-immunized mice seroconverted and reacted with MAP1 in antigen blots. Splenocytes from immunized mice, but not from control mice immunized with VCL1010 DNA (plasmid vector, Vical, San Diego) proliferated in response to recombinant MAP1 and *C. ruminantium* antigens in *in vitro* lymphocyte proliferation tests. These proliferating cells from mice immunized with VCL1010/MAP1 DNA secreted IFN-gamma and IL-2 at concentrations ranging from 610 pg/ml and 152 pg/ml to 1290 pg/ml and 310 pg/ml, respectively. In experiments testing different VCL1010/MAP1 DNA vaccine dose regimens (25-100 µg/dose, 2 or 4 immunizations), survival rates of 23% to 88% (35/92 survivors/total in all VCL1010/MAP1 immunized groups) were observed on challenge with 30LD50 of *C. ruminantium*. Survival rates of 0% to 3% (1/144 survivors/total in all control groups) were recorded for control mice immunized similarly with VCL1010 DNA or saline. Accordingly, in a specific embodiment, the subject invention

concerns the discovery that the gene encoding the MAP1 protein induces protective immunity as a DNA vaccine against rickettsial disease.

The nucleic acid sequences described herein have other uses as well. For example, the nucleic acids of the subject invention can be useful as probes to identify complementary sequences within other nucleic acid molecules or genomes. Such use of probes can be applied to identify or distinguish infectious strains of organisms in diagnostic procedures or in rickettsial research where identification of particular organisms or strains is needed. As is well known in the art, probes can be made by labeling the nucleic acid sequences of interest according to accepted nucleic acid labeling procedures and techniques. A person of ordinary skill in the art would recognize that variations or fragments of the disclosed sequences which can specifically and selectively hybridize to the DNA of rickettsia can also function as a probe. It is within the ordinary skill of persons in the art, and does not require undue experimentation in view of the description provided herein, to determine whether a segment of the claimed DNA sequences is a fragment or variant which has characteristics of the full sequence, *e.g.*, whether it specifically and selectively hybridizes or can confer protection against rickettsial infection in accordance with the subject invention. In addition, with the benefit of the subject disclosure describing the specific sequences, it is within the ordinary skill of those persons in the art to label hybridizing sequences to produce a probe.

Various degrees of stringency of hybridization can be employed. The more severe the conditions, the greater the complementarity that is required for duplex formation. Severity of conditions can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*, Stockton Press, New York, NY., pp. 169-170.

Examples of various stringency conditions are provided herein. Hybridization of immobilized DNA on Southern blots with <sup>32</sup>P-labeled gene-specific probes can be performed by standard methods (Maniatis *et al.* (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York.). In general, hybridization and subsequent washes can be carried out under moderate to high stringency conditions that allow for detection of target sequences with homology to the exemplified polynucleotide sequence. For double-stranded DNA gene probes, hybridization can be carried out overnight at 20-25° C below the melting temperature (*T<sub>m</sub>*) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula (Beltz *et al.*

*et al.* [1983] *Methods of Enzymology*, R. Wu, L. Grossman and K. Moldave [eds.] Academic Press, New York 100:266-285).

$T_m = 81.5^{\circ}\text{C} + 16.6 \text{ Log}[\text{Na}^+] + 0.41(\%G+C) - 0.61(\%\text{formamide}) - 600/\text{length of duplex in base pairs.}$

5 Washes are typically carried out as follows:

- (1) twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash);
- (2) once at  $T_m - 20^{\circ}\text{C}$  for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

10 For oligonucleotide probes, hybridization can be carried out overnight at  $10-20^{\circ}\text{C}$  below the melting temperature ( $T_m$ ) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA.  $T_m$  for oligonucleotide probes can be determined by the following formula:

$T_m (^{\circ}\text{C}) = 2(\text{number T/A base pairs}) + 4(\text{number G/C base pairs})$  (Suggs *et al.* [1981] *ICN-UCLA Symp. Dev. Biol. Using Purified Genes*, D.D. Brown [ed.], Academic Press, New York, 23:683-693).

Washes can be carried out as follows:

- (1) twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash);
- 20 (2) once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment >70 or so bases in length, the following conditions can be used:

- |           |                                       |
|-----------|---------------------------------------|
| Low:      | 1 or 2X SSPE, room temperature        |
| 25 Low:   | 1 or 2X SSPE, $42^{\circ}\text{C}$    |
| Moderate: | 0.2X or 1X SSPE, $65^{\circ}\text{C}$ |
| High:     | 0.1X SSPE, $65^{\circ}\text{C}$ .     |

Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probe sequences of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions and deletions can be produced in a given

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polynucleotide sequence in many ways, and these methods are known to an ordinarily skilled artisan. Other methods may become known in the future.

It is also well known in the art that restriction enzymes can be used to obtain functional fragments of the subject DNA sequences. For example, *Bal31* exonuclease can be conveniently used for time-controlled limited digestion of DNA (commonly referred to as "erase-a-base" procedures). See, for example, Maniatis *et al.* (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York; Wei *et al.* (1983) *J. Biol. Chem.* 258:13006-13512.

In addition, the nucleic acid sequences of the subject invention can be used as molecular weight markers in nucleic acid analysis procedures.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

#### Example 1

A nucleic acid vaccine construct was tested in animals for its ability to protect against death caused by infection with the rickettsia *Cowdria ruminantium*. The vaccine construct tested was the MAP1 gene of *C. ruminantium* inserted into plasmid VCL1010 (Vical, San Diego) under control of the human cytomegalovirus promoter-enhancer and intron A. In this study, seven groups containing 10 mice each were injected twice at 2-week intervals with either 100, 75, 50, or 25 µg VCL1010/MAP1 DNA (V/M in Table 1 below), or 100, 50 µg VCL1010 DNA (V in Table 1) or saline (Sal.), respectively. Two weeks after the last injections, 8 mice/group were challenged with 30LD50 of *C. ruminantium* and clinical symptoms and survival monitored. The remaining 2 mice/group were not challenged and were used for lymphocyte proliferation tests and cytokine measurements. The results of the study are summarized in Table 1, below:

**Table 1**

	100 µg V/M	75 µg V/M	50 µg V/M	25 µg V/M	100 µg V	50 µg V	Sal.
Survived	5	7	5	3	0	0	0
Died	3	1	3	5	8	8	8

The VCL1010/MAP1 nucleic acid vaccine increased survival on challenge in all groups, with a total of 20/30 mice surviving compared to 0/24 in the control groups.

This study was repeated with another 6 groups, each containing 33 mice (a total of 198 mice). Three groups received 75 µg VCL1010/MAP1 DNA or VCL1010 DNA or saline (4 injections in all cases). Two weeks after the last injection, 30 mice/group were challenged with 30LD50 of *C. ruminantium* and 3 mice/group were sacrificed for lymphocyte proliferation tests and cytokine measurements. The results of this study are summarized in Table 2, below:

Table 2						
	V/M 2 inj.	V 2 inj.	Sal. 2 inj.	V/M 4 inj.	V 4 inj.	Sal. 4 inj.
Survived	7	0	0	8	0	1
Died*	23	30	30	22	30	29

\*In mice that died in both V/M groups, there was an increase in mean survival time of approximately 4 days compared to the controls ( $p < 0.05$ ).

Again, as summarized in Table 2, the VCL1010/MAP1 DNA vaccine increased the numbers of mice surviving in both immunized groups, although there was no apparent benefit of 2 additional injections. In these two experiments, there were a cumulative total of 35/92 (38%) surviving mice in groups receiving the VCL1010/MAP1 DNA vaccine compared to 1/144 (0.7%) surviving mice in the control groups. In both immunization and challenge trials described above, splenocytes from VCL1010/MAP1 immunized mice, but not from control mice, specifically proliferated to recombinant MAP1 protein and to *C. ruminantium* in lymphocyte proliferation tests. These proliferating splenocytes secreted IL-2 and gamma-interferon at concentrations up to 310 and 1290 pg/ml respectively. These data show that protection against rickettsial infections can be achieved with a DNA vaccine. In addition, these experiments show MAP1-related proteins as vaccine targets.

#### Example 2 – Cloning and sequence analysis of MAP1 homologue genes of *E. chaffeensis* and *E. canis*

Genes homologous to the major surface protein of *C. ruminantium* MAP1 were cloned from *E. chaffeensis* and *E. canis* by using PCR cloning strategies. The cloned segments represent a 4.6 kb genomic locus of *E. chaffeensis* and a 1.6 kb locus of *E. canis*. DNA sequence generated from these clones was assembled and is presented along with the deduced amino acid

sequence in Figures 2A-2B (SEQ ID NOs. 7-11 and 14-18) and Figure 2C (SEQ ID NOs. 12-13 and 19-20). Significant features of the DNA include five very similar but nonidentical open reading frames (ORFs) for *E. chaffeensis* and two very similar, nonidentical ORFs for the *E. canis* cloned locus. The ORFs for both *Ehrlichia* spp. are separated by noncoding sequences ranging from 264 to 310 base pairs. The noncoding sequences have a higher A+T content (71.6% for *E. chaffeensis* and 76.1% for *E. canis*) than do the coding sequences (63.5% for *E. chaffeensis* and 68.0% for *E. canis*). A G-rich region -200 bases upstream from the initiation codon, sigma-70-like promoter sequences, putative ribosome binding sites (RBS), termination codons, and palindromic sequences near the termination codons are found in each of the *E. chaffeensis* noncoding sequences. The *E. canis* noncoding sequence has the same feature except for the G-rich region (Figure 2C; SEQ ID NOs. 12-13 and 19-20).

Sequence comparisons of the ORFs at the nucleotide and translated amino acid levels revealed a high degree of similarity between them. The similarity spanned the entire coding sequences, except in three regions where notable sequence variations were observed including some deletions/insertions (Variable Regions I, II and III). Despite the similarities, no two ORFs are identical. The cloned ORF 2, 3 and 4 of *E. chaffeensis* have complete coding sequences. The ORF1 is a partial gene having only 143 amino acids at the C-terminus whereas the ORF5 is nearly complete but lacks 5-7 amino acids and a termination codon. The cloned ORF2 of *E. canis* also is a partial gene lacking a part of the C-terminal sequence. The overall similarity between different ORFs at the amino acid level is 56.0% to 85.4% for *E. chaffeensis*, whereas for *E. canis* it is 53.3%. The similarity of *E. chaffeensis* ORFs to the MAP1 coding sequences reported for *C. ruminantium* isolates ranged from 55.5% to 66.7%, while for *E. canis* to *C. ruminantium* it is 48.5% to 54.2%. Due to their high degree of similarity to MAP1 surface antigen genes of *C. ruminantium* and since they are nonidentical to each other, the *E. chaffeensis* and *E. canis* ORFs are referred to herein as putative Variable Surface Antigen (VSA) genes. The apparent molecular masses of the predicted mature proteins of *E. chaffeensis* were 28.75 kDa for VSA2, 27.78 for VSA3, and 27.95 for VSA4, while *E. canis* VSA1 was slightly higher at 29.03 kDa. The first 25 amino acids in each VSA coding sequence were eliminated when calculating the protein size since they markedly resembled the signal sequence of *C. ruminantium* MAP1 and presumably would be absent from the mature protein.

The amino acid sequence derived from the cloned *E. chaffeensis* MAP1-like gene, and alignment with the corresponding genes of *C. ruminantium* and *A. marginale* is shown in Figure 1.

Example 3

A further aspect of the subject invention are five additional genes which give protection when formatted as DNA vaccines. These genes are *Cowdria ruminantium* map 2, *Cowdria ruminantium* lhworf3, *Cowdria ruminantium* 4hworf1, *Cowdria ruminantium* 18hworf1, and *Cowdria ruminantium* 3gdorf3. The DNA and translated amino acid sequences of these five genes are shown in SEQ ID NOS. 25-34.

There is published information showing that gene homologs of all five genes are present in other bacteria. For example, a homolog of map2 is present in *Anaplasma marginale*, a homolog of lhworf3 is present in *Brucella abortus*, homologs of 4hworf1 are present in *Pseudomonas aeruginosa* and *Coxiella burnetii*, and homologs of 18hworf1 are present in *Coxiella burnetii* and *Rickettsia prowazekii*. This can be revealed by a search of DNA and protein databases with standard search algorithms such as "Blast". Based on the protective ability of these genes against *Cowdria ruminantium* and their presence in other bacterial pathogens, the subject invention further concerns the use of these genes, their gene products, and the genes and gene products of the homologs as vaccines against bacteria. This includes their use as DNA or nucleic acid vaccines or when formulated in vaccines employing other methods of delivery, e.g., recombinant proteins or synthetic peptides in adjuvants, recombinant live vector delivery systems such as vaccinia (or other live viruses) or *Salmonella* (or other live bacteria). These methods of delivery are standard to those familiar with the field. This also includes vaccines against heartwater disease, vaccines against rickettsial diseases in general and vaccines against other bacteria containing homologs of these genes.

Table 3 shows the protective ability of the 5 genes against death from *Cowdria ruminantium* challenge in mice. Genes were inserted into VR1012 according to the manufacturers instructions (Vical, San Diego) and challenge studies were conducted as described in Example 1. N-terminal sequences which putatively encoded prokaryotic signal peptides were deleted because of the potential for their affects on expression and and immune responses in eukaryotic expression systems or challenged animals. The inserts were as follows: map2, SEQ ID NO. 25, beginning at base 46; 18hworf1, SEQ ID NO. 31, beginning at base 67; 3gdorf3, SEQ ID NO. 33, beginning at base 79; lhworf3, SEQ ID NO. 27, beginning at base 76; and 4hworf1, SEQ ID NO. 29, beginning at base 58.

Table 3

DNA Construct	MWT Size	Survival Rate				
		Vaccinated		Control		P value
TMMAP 2	21 kd	9/28*	32%	0/29	0%	0.004
MB18HWORF1	28 kd	10/30*	33%	1/27	4%	0.021
AM3GDORF3	16 kd	7/26	27%	1/27	4%	0.060
TM1HWORF3	36 kd	8/29	28%	2/30	7%	0.093
TM4HWORF1	19 kd	10/30*	33%	2/30	7%	0.054

Control - VR1012 DNA vector plasmid only

\*Statistically significant difference (Fisher's Exact test)

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

Claims

1           1. A composition comprising a polynucleotide which encodes a polypeptide having the  
2           characteristic of eliciting an immune response protective against disease or death caused by a  
3           ricketsial pathogen.

1           2. The composition, according to claim 1, wherein said rickettsial pathogen is selected  
2           from the group consisting of *Rickettsia* spp., *Ehrlichia* spp., *Anaplasma* spp., and *Cowdria* spp.

1           3. The composition, according to claim 1, wherein said polypeptide has an amino acid  
2           sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6,  
3           SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NOS. 16-20, SEQ ID NO. 23, SEQ ID NO. 24, SEQ  
4           ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, homologs  
5           thereof, and immunogenic fragments thereof.

1           4. The composition, according to claim 1, wherein said polynucleotide has a nucleic  
2           acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO.  
3           5, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NOS. 9-13, SEQ ID NO. 21, SEQ ID NO. 22, . SEQ  
4           ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, homologs  
5           thereof, and fragments thereof which encode immunogenic polypeptides.

1           5. The composition, according to claim 4, wherein said polynucleotide has a nucleic  
2           acid sequence of SEQ ID NO. 3, or a fragment thereof.

1           6. The composition, according to claim 1, wherein said polynucleotide further  
2           comprises a nucleic acid vaccine vector.

1           7. The composition, according to claim 1, further comprising a pharmaceutically  
2           acceptable carrier.

1           8. A polynucleotide encoding a polypeptide having an amino acid sequence selected  
2           from the group consisting of SEQ ID NO. 4, SEQ ID NOS. 14-20, SEQ ID NOS. 23-24, SEQ  
3           ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, and fragments  
4           thereof.

1           9. The polynucleotide, according to claim 8, said polynucleotide having a nucleic acid  
2           sequence selected from the group consisting of SEQ ID NO. 3, SEQ ID NOS. 7-13, SEQ ID  
3           NOS. 21-22, SEQ ID NOS. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, and SEQ ID  
4           NO. 33.

1           10. A method for protecting a susceptible host against disease or death caused by a  
2           ricketsial pathogen, said method comprising administering an effective amount of a  
3           polynucleotide encoding polypeptide having the characteristic of eliciting an immune response  
4           protective against said rickettsial pathogen.

1           11. The method, according to claim 10, wherein said rickettsial pathogen is selected  
2           from the group consisting of *Rickettsia* spp., *Ehrlichia* spp., *Anaplasma* spp., and *Cowdria* spp.

1           12. The method, according to claim 10, wherein said polypeptide has an amino acid  
2           sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6,  
3           SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NOS. 16-20, SEQ ID NO. 23, SEQ ID NO. 24, SEQ  
4           ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, or homologs  
5           thereof and immunogenic fragments thereof.

1           13. The method, according to claim 10, wherein said polynucleotide has a nucleic acid  
2           sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO. 5,  
3           SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NOS. 9-13, SEQ ID NO. 21, SEQ ID NO. 22, SEQ ID  
4           NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, and SEQ ID NO. 33.

1           14. The method, according to claim 13, wherein said polynucleotide has the nucleic acid  
2           sequence of SEQ ID NO. 1.

1           15. The method, according to claim 13, wherein said polynucleotide has the nucleic acid  
2           sequence of SEQ ID NO. 3.

1           16. The method, according to claim 13, wherein said polynucleotide has the nucleic acid  
2           sequence of SEQ ID NO. 5.

1 17. The method, according to claim 10, wherein said nucleic acid further comprises an  
2 appropriate nucleic acid vector.

1 18. The method, according to claim 10, wherein said composition further comprises a  
2 pharmaceutically acceptable carrier.

1 19. The method, according to claim 10, which further comprises administration to said  
2 host of said polypeptide encoded by said polypeptide.

1 20. A method for detecting, in a human or animal, antibodies associated with infection  
2 by *Ehrlichia*, wherein said method comprises contacting a biological fluid from said human or  
3 animal with a polypeptide selected from the group consisting of SEQ ID NO. 4, SEQ ID NOS.  
4 14-20, SEQ ID NOS. 23-24, SEQ ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO.  
5 32, SEQ ID NO. 34, and homologs and fragments thereof.

1 21. A method of detecting the presence of rickettsial nucleic acids comprising  
2 contacting a sample suspected of containing rickettsial nucleic acids with a composition  
3 comprising a labeled polynucleotide which encodes a polypeptide having the characteristic of  
4 eliciting an immune response protective against disease or death caused by a rickettsial  
5 pathogen, allowing for the formation of a hybridization complex and detecting said label.

1 22. The composition, according to claim 21, wherein said rickettsial pathogen is  
2 selected from the group consisting of *Rickettsia* spp., *Ehrlichia* spp., *Anaplasma* spp., and  
3 *Cowdria* spp.

1 23. The composition, according to claim 21, wherein said polypeptide has an amino acid  
2 sequence selected from the group consisting of SEQ ID NO. 2, SEQ ID NO. 4, SEQ ID NO. 6,  
3 SEQ ID NO. 14, SEQ ID NO. 15, SEQ ID NOS. 16-20, SEQ ID NO. 23, SEQ ID NO. 24, SEQ  
4 ID NO. 26, SEQ ID NO. 28, SEQ ID NO. 30, SEQ ID NO. 32, SEQ ID NO. 34, and homologs  
5 and immunogenic fragments thereof.

1 24. The composition, according to claim 21, wherein said polynucleotide has a nucleic  
2 acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 3, SEQ ID NO.  
3 5, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NOS. 9-13, SEQ ID NO. 21, SEQ ID NO. 22, SEQ

- 4 ID NO. 25, SEQ ID NO. 27, SEQ ID NO. 29, SEQ ID NO. 31, SEQ ID NO. 33, homologs  
5 thereof, and fragments thereof which encode immunogenic polypeptides.

## FIG. 1A

*C.r.* ATGAATTGCAAGAAATTTTA-----TCACAAGTACACTAATATCATTAGTG  
*E.c.* ATGAATTACAAAAAAGTTTCA-----TAACAGCG-ATTGATATCATTAATA  
*A.m.* ATGAATTACAGAGAAATTGTTTACAGGGGGCCTG-TCAGCAGCC-ACAGTCTGGCCTGCT  
 \*\*\*\*\* \*\* \*\* \* \*\* \*

*C.r.* TCATTTT--TACCTGGTGTCTCCTTTTCTGATGTAATACAGGAAGACAGCAACCCAGCAG  
*E.c.* TCCTTCTCTTACCCTGGAGTATCATTTTCCGACCCCAAGGAGGTAGTGCTCA---TTAACG  
*A.m.* CCCTACTTGTAGTGGGCCGTAGTGGCATCTCCCATGAGTCACGAAGTGGCTTCTGAAG  
 \* \* \* \* \* \*\* \* \* \* \*

*C.r.* GCAGTGTTACATTAGCGCAAAATACATGCCAACTGCATCACATTTTGGTAAATGTCAA  
*E.c.* GTAAATTTCTACATCAGTGGAAATACGATGCCAAGGCTTCGCATTTTGGAGTATCTCTG  
*A.m.* GGGAGTAATGGGAGGTAGCTTTTACGTGGTGCGGCCT-ACAGCCCAAGCATTTCCCTTCT  
 \* \* \* \* \* \*\* \* \* \*

*C.r.* TCAAAGAAGATTCAAAAAATACTCAAACGGTATTTGGTCTAAAAAAGATTGGGATGGCG  
*E.c.* CTAAGGAAGAAAGAAATACAAACAGTTGGAGTGTTGGACTGAAGCAAAATTTGGACGGAA  
*A.m.* GTTACCTCGTTCGACATGCGTGAGTCAAGCAAGAGACCTCA--TACGTTAGAGGCTATG  
 \* \* \* \* \* \*\* \* \* \*

*C.r.* TTAAACACCATCAGATTCTAGCAATACTAATTCTACAATTTTACTGAAAAAGACTATT  
*E.c.* GCGCAATATC--CAACTCCTCCCCAAACGA-----TGTATTCACTGTCTCAAAATTATT  
*A.m.* ACAAGAGCATTGCAACGATTGATGTGAGTGTGCCAGCAAACTTTTCCAAATCTGGCTACA  
 \* \*\* \* \* \*

*C.r.* CTTTCAGATATGAAAAACAATCCGTTTTTTAGGTTTCGCTGGAGCAATTGGGTACTCAATGA  
*E.c.* CATTAAATATGAAAAACAACCCGTTTTTTAGGTTTTCGAGAGCTATTGGTTACTCAATGG  
*A.m.* CTTTGGCCTTCTCTAAAAACTTAATCACGTCCTTCGACGGCGCTGTGGATATCTCTCTGG  
 \* \* \* \* \* \*\* \* \* \*

## FIG. 1B

<i>C.r.</i>	ATGGACCAAGAAATAGAGTTCGAAGTATCCTATGAAACTTTTGATGTAAAAACCTAGGTG	
<i>E.c.</i>	ATGGTCCAAAGAAATAGAGCTTGAAGTATCTTATGAACATTTGATGTAAAAATCAAGGTA	
<i>A.m.</i>	GAGGAGCCAGAGTGGAATTGGGAAGCGAGCTACAGAAGGTTTGCTACTTTGGCGGACGGGC	**
	** * ** * ** * ** * ** * ** * ** *	**
<i>C.r.</i>	GCAACTATAAAACAACGCACACATGTACTGTGCTTTAGATACAGCAGCACAATAAGCA	
<i>E.c.</i>	ACAATTATAAGAAATGAAGCACATAGATATTGTGCTCTATCCCATAACTCAGCAGCAGACA	
<i>A.m.</i>	AGTACGCCAAAAGTG-----GTGCGGAATCTCTGGCAGCTATTACCCGCG	*
	* ** *	*** *
<i>C.r.</i>	CTAATGGCGCAGGATTAACTACATCTGTTATGTTAAAAACGAAAATTTAACAAATATAT	
<i>E.c.</i>	TGAGTAGTGCAAG---TAATAATTTTGCTCTTTCTAAAAAATGAAGGATTACTTGACATAT	
<i>A.m.</i>	ACGCTAACATTACTGAGACCAATTACTTCGTAGTCAAAAATGATGAAATCACAACACCT	*
	* * * * *	* * * * *
<i>C.r.</i>	CATTAATGTTAAATGCGTGTTATGATATCATGCTTGATGGAATACCAGTTTCTCCATATG	
<i>E.c.</i>	CATTTATGCTGAACGCATGCTATGACGTAGTAGGCGAAGGCATACCTTTTCTCCTTATA	
<i>A.m.</i>	CAGTCATGTTAAATGGCTGCTATGACGTGCTGCACACAGATTTACCTGTGTCCTCCCGTATG	**
	** * ** * ** * ** * ** * *	*** * * * * **
<i>C.r.</i>	TATGTGCAGGTATTGGCACTGACTTAGTGTCAGTAATTAATGCTACAAATCCTAAATTAT	
<i>E.c.</i>	TATGCGCAGGTATCGGTACTGATTAGTATCCATGTTTGAAGCTACAAATCCTAAATTT	
<i>A.m.</i>	TATGTGCCGGGATAGGCGCAAGCTTTGTTGACATCTCTAAGCAAGTAACCCACAAAGCTGG	***
	*** ** * ** * ** * ** * ** *	** * * * *
<i>C.r.</i>	CTTATCAAGGAAAGCTAGGCATAAGTTACTCAATCAATTCTGAAGCTTCTATCTTTATCG	
<i>E.c.</i>	CTTACCAAGGAAAGTTAGGTTTAAGCTACTCTATATAAGCCCGAAGCTTCTGTGTTTATG	
<i>A.m.</i>	CCTACAGGGGCAAGTTGGGATTAGCTACCAGTTTACTCCGGAAATATCCTTGGTGGCAG	*
	* ** * ** * ** * ** * ** *	* ** * ** *

## FIG. 1C

<i>C.r.</i>	GTGGACATTTCCATAGAGTTATAGGTAATGAATTTAAAGATATTGCTACCTTAAATAATAT	
<i>E.c.</i>	GTGGGCACCTTTCATAAGGTAATAGGGAACGAATTTAGAGATATTCCCTACTATAATACCTA	
<i>A.m.</i>	GTGGGTTCTACCAACGGGCTATTTTGATGAGTCTTACAAGGACATTTCCCGCACACAACAGTG	*
	**** * ** * * * * * * * * * * *	
<i>C.r.</i>	TTACTTCAAAAACAGGAATATCTAATCCCTGGCTTTGCATCAGCAACACTTGATGTTGTC	
<i>E.c.</i>	CTGGATCAACACTTGCAGGAAAGGAACTACCCCTGCAATAGTAATACTGGATGTATGCC	
<i>A.m.</i>	TAAAGTTCTCTGGAGAAGCAAAA-----GCCTCAGTCAAAGCGCATATTGCTG	*
	* * * * * ** * * * * *	
<i>C.r.</i>	ACTTTGGTATAGAAATTGGAGGAAGGTTTGTAATTTTAA---	
<i>E.c.</i>	ACTTTGGAAATAGAAATGGGAGGAAGGTTTAA-----	
<i>A.m.</i>	ACTACGGCTTTAACCTTGGAGCAAGATTCCCTGTTTCAGCTAA	
	*** ** * * * **** *** **	

1 ggaatgaattcagggacattctactcttaaaagcgtttgctacaccatcatctgcagcta  
 N E F R D I S T L K A F A T P S S A A T  
 61 ctccagacttagcaacagtaacactgagtggtgtcactctggagtagaacttggaggaa  
 P D L A T V T L S V C H F G V E L G G R  
 121 gacttaactcttaattcttatttgcacatgcttaaaaaataatctaaacttgccttcatt  
 F N F \*  
 181 actgctacaaataaataaaatagtggaacaaatgtagcaataagagggggggggggga  
 241 ctaacttactatctgccatatacccttactaccacttacactaaataatctgacaaatacaa  
 301 cagctcttggagaaataaacaatatttaaatcttcttacaacaaaccatttatatcttgc  
 -35  
 361 actaaaaactagcttatcaacttgccttttacattgtaggttactactgttaatttgcctt  
 -10  
 421 cactattctcaggtgtaactgaactgcgaaaaatcttttatacaactgcatttaacatta  
 RBS M N C E K F F I T T A L T L  
 481 ctaatgtccttcttactctggaatcatcactttctgatccagtagcaggatgacaacattagt  
 L M S F L P G I S L S D P V Q D D N I S  
 541 ggttaattctcatcagtggaagtatatgccaaagcgttgcgattttggagttttctt  
 G N F Y I S G K Y M P S A S H F G V F S  
 601 gccaaaggaagaaagaaacacacagctggagtagtttggaaatagagcaagattgggataga  
 A K E E R N T T V G V F G I E Q D W D R  
 661 tgtgtaatatctagaaccacttttaagcgatatactaccgttccaaaattatctatctaa  
 C V I S R T T L S D I F T V P N Y S F K  
 721 catgaaataatcttattctcaggatttgcaggagctattggtactcaatggatggccca  
 Y E N N L F S G F A G A I G Y S M D G P  
 781 agaataagagcttgaagtagcttgaagcattctgattgtaaaaaatcaaggtaacattat  
 R I E L E V S Y E A P D V K N Q G N N Y  
 841 aagaacgaagcagatagatattatgctctgtccatcttctcggcacagagacacagata  
 K N E A H R Y Y A L S H L L G T E T Q I  
 901 gatggtgcaggcagtgctctgtcttcttaataaaatgaaggactacttgataaaatcatt  
 D G A G S A S V F L I N E G L L D K S P  
 961 atgctgaacgcatgttatgtagtaaaagtgaaagcacaactcttctctcttatatagt  
 M L N A C Y D V I S E G I P F S P Y I C  
 1021 gcagggtattggtattgatttagtattccatcttgaagctataaaatcttcttctat  
 A G I G I D L V S M F E A I N P K I S Y  
 1081 caaggaaaaattaggtcttaagttaccctataagcccaagagcttctgtgcttattggtgga  
 Q G K L G L S Y P I S P E A S V F I G G  
 1141 cactttcacaaggtgataggaacgaatttagagatattcttactatgatccttagtgaa  
 H F H K V I G N E F R D I P T M I P S E  
 1201 tcagcgttgcaggaaaaggaaactaccctgcaatagtaacactggacgtgtcttacttt  
 S A L A G K G N Y P A I V T L D V F Y F  
 1261 ggcatagaacttggaggaagggttaacttccaaactttgattattgocacaaataaaaa  
 G I E L G G R F N F Q L \*  
 1321 atagtggcaaaagaatgtagcaataagaggggggggggaactaaattattatttgc  
 1381 atatcccttactaccacttacaccaataatctgacaaatacaacaggttcaaacaaagg  
 1441 aaacaattcttaatttgccttcttgagaaccattgatatcttatactaaaaacttagctta  
 -35  
 1501 taacttgccttttacattgcagcttctactattgttaattttatcttactatttttaggtgta  
 RBS  
 1561 atatgaattgcaaaaaatcttttatacaactgcatttagtatcactaatgtcctttctac  
 M N C K K F F I T T A L V S L M S F L P  
 1621 ctggaatatcattttctgacccagtgcaaggtgacaataattagtggtaacttctatgta  
 G I S F S D P V Q G D N I S G N F Y V S  
 1681 gtggcaagtagatgccaaagcgttgcgatttttggcatgtttctgccaagaagaaaaaa  
 G K Y M P S A S H F G M P S A K E E K N  
 1741 atcctactgttgcattgtatggcttaaaacaagattgggaagggattagctcatcaagtc  
 P T V A L Y G L K Q D W E G I S S S H  
 1801 acaatgataatcatttcaataacaagggctattcatcttaaatatgaaaataacccatttt  
 N D N H P N N K G Y S F K Y E N N P F L  
 1861 tagggcttgcaggagctattggttattcaatgggtggtccaaagagtagagtttgaagtgt  
 G F A G A I G Y S M G G P R V E F E V S  
 1921 cctatgaaacatttgacgttaaaaaatcagggttaataactataaaaaatgatgctcacagat  
 Y E T F D V K N Q G N N Y K N D A H R Y  
 1981 actgtgctttaggtcaacaagacacagcggaataacctaacttagtaaatagctactgt  
 C A L G Q Q D N S G I P K T S K Y V L L  
 2041 taaaaagcgaaggattgcttgacatatcattttatgctaaatgcattgctatgataataaa  
 K S E G L L D I S F M L N A C Y D I N  
 2101 acgagagcataccttctgtctcttctacatatgtgcagggtgttggtactgatttaatatcca  
 E S I P L S P Y I C A G V G T D L I S M  
 2161 tcttgaagctataaaatcttcttcaaaatcttcttcaaaaggaaggttaggtctaaagtactcta  
 F E A T N P K I S Y Q G K L G L S Y S I  
 2221 taaacccagaagcttctgtacttatttgggtggacattttcataaggtgataggaaacgaat  
 N P E A S V F I G G H F H K V I G N E F  
 2281 ctagggaacttctactctgaaagcatttgcctacgtcatcagctactccagatctagcaa  
 R D I P T L K A F V T S S A T P D L A I

FIG. 2A

2341 tagcaacactaagtggtatgtcatttttggaaatagaacttggaggaaggttttaacttttaac  
 V T L S V C H F G I E L G G R F N F \*  
 2401 tttgttacttggccacatgttataaaataatcttaacttgtttttcattttgtctacagtaaac  
 2461 aaaaatagtggtcaaaaagaatgtagcaataagaaggggggggggggactaaattgtctattt  
 2521 accatacccccttatttatccactttacactaaataacttgacaaatacaaacagctttctgga  
 2581 aaaaacaaacacactttaaattttctctttacaaaaaccattttatattcttctgactaaaaacta  
 -15  
 2641 gctttataaccttgtttttacacttgaagcttctactatttgcataattttttcactatttttag  
 -10  
 2701 gtgcataatgaattgcaaaaaattttttataacaactacatttagtatcgctaatgtctctt  
 RBS M N C K K F F I T T T L V S L M S P  
 2761 cttacctgggaatcatcttttctgagtgacagacagaacgaatgtttggtggtaattttcta  
 L P G I S F S D A V Q N D N V G G N F Y  
 2821 tatcagtgggaaatctgtaccaagtggtttcacattttggcggtattctctgctaaacagga  
 I S G K Y V P S V S H F G V F S A K Q E  
 2881 aagaaatcaacaacatcggaagtatttggattaaagcaagattgggagtcagcagcaatcatc  
 R N T T I G V F G L K Q D W D G S T I S  
 2941 caaaaattctctcagaaaatcatctttaaagttccaaaattatttcaatttcaaatatcaaaaataa  
 K N S P E N T F N V P N Y S F K Y E N N  
 3001 tccattttctaggttttgcaggagctgttgggttattttaatgaatgttccaaagtaagatt  
 P P L G P A G A V G Y L M N G P R I E L  
 3061 agaaatgtctctatgaacatttggatgtgaaaaaccagggttaataactataagaacgatgc  
 E M S Y E T F D V K N Q G N N Y K N D A  
 3121 tcacaatatctatgcttttaacccataacagtggggggaaagctaaagcaatgcaggtgataa  
 H K Y Y A L T H N S G G K L S N A G D K  
 3181 gtttggttttttcaaaaaatgaaggactacttgatatactactttatgttgaatgcattgcta  
 F V F L K N E G L L D I S L M L N A C Y  
 3241 tgatgtaataagtgaaaggaatacctttctctcttactatgtgcaggtgttggtagtga  
 D V I S E G I P F S P Y I C A G V G T D  
 3301 tttatattccatgttttgaagctataaaaaccttaaaattttcttatcaaggaaagtttaggttt  
 L I S M F E A I N P K I S Y Q G K L G L  
 3361 gagttactccataagcccgagaagcttctgtttttgttgggtggacattttcataaggtgac  
 S Y S I S P E A S V F V G G H F H K V I  
 3421 aggggaatgaatttcagagatattcctgtctatgatccccagtcactcaactctcacaggtaa  
 G N E F R D I P A M I P S T S T L T G N  
 3481 tcactttactatagtaacactaagtgtatgccacttttggagtggaacttggaggaaggtt  
 H F T I V T L S V C H F G V E L G G R F  
 3541 taactttttaatttttatttgcacatgttataaaataatctaaacttgttttttattattg  
 N F: \*  
 3601 ctgcaggtaaatataaataatagtggtgcaaaaagaatgtagcaataagaaggggggggggactag  
 3661 tttataagtgctgtttttttctaccttttacacatgatactatacttaaacaggttttttttgc  
 3721 tactacttacctgacgtaataatcttaaaattttcttacaaggttaccgatactttttatac  
 -35  
 3781 aaaaattttattttctgacttgccttttttatatgacactttcactatttgcataattttatttgc  
 -10  
 3841 actattttaggtttatatatgaattacaaaaaagtttttataacaagtgcatgtgatattatta  
 RBS M N Y K K V F I T S A L I S L  
 3901 atattctctctacctggagtgatctttttccgacccagcaggttagtggtatttaacggtaat  
 I S S L P G V S F S D P A G S G I N G N  
 3961 tttcacatcagtggaataatcatgccaagtgtcttcgcattttggagtgattctctgctaag  
 F Y I S G K Y M P S A S H F G V F S A K  
 4021 gaagaaagaaatcaaacagtttggagtggtttggactgaagcaaaaatgggacggaagcgca  
 E E R N T T V G V F G L K Q N W D G S A  
 4081 atatcccaactctctcccaaacgatgtattcactgtctcaaaattatttcaatttcaaatatcaa  
 I S N S S P N D V F T V S N Y S F K Y E  
 4141 aacaacccgcttttttaggttttgcaggagctatttgggttactcaatggatgggtccaagaata  
 N N P F L G F A G A I G Y S M D G P R I  
 4201 gagcttgaagtattcttatgaacatttggatgtaaaaaatcaaggtaacaattataagaat  
 E L E V S Y E T F D V K N Q G N N Y K N  
 4261 gaagcacatagatatttgtctctatcccaataactcagcagcagacatgagtagtgcaagt  
 E A H R Y C A L S H N S A A D M S S A S  
 4321 aataattttgtcttttcaaaaaatgaaggattacttgacatattcatttattgctgaacgca  
 N N F V F L K N E G L L D I S F M L N A  
 4381 tgctatgacgtagtaggcgaaggcatacctttttctctcttatatgctgcaggtatcggt  
 C Y D V V G E G I P F S P Y I C A G I G  
 4441 actgatttagtatccatgtttgaagctacaaatcttcaaaattttctcaaggaaggtta  
 T D L V S M F E A T N P K I S Y Q G K L  
 4501 ggtttaagctactctataagcccgagaagcttctgtgtttatttgggtgggcaactttcataag  
 G L S Y S I S P E A S V F I G G H F H K  
 4561 gtaatagggaacgaatttttagagatatttctactataatacctactggatcaaacacttgca  
 V I G N E F R D I P T I I P T G S T L A  
 4621 ggaaaaggaaactaccctgcaatagtaatactggatgtatgccacttttgaatagaatg  
 G K G N Y P A I V I L D V C H F G I E M  
 4681 gga  
 G

FIG. 2B

```

1 tggtgtaaatatgaaatatataaaaaaacttttacagtaactgcattagttattattaacttc
   RBS      M K Y K K T F T V T A L V L L T S
61 ctttacacattttatatttttatagtcacgacgtgcsagtacaattcacacatttcta
   F T H F I P F Y S P A R A S T I H N F Y
121 cattagtggaataatataatgccaaacagcgtcacatttttgggaatttttcagctaaagaaga
   I S G K Y M P T A S H F G I F S A K E E
181 acaaagttttactaagggtattagttgggttagatcaacgattatcacataatattatataa
   Q S F T K V L V G L D Q R L S H N I I N
241 caataatgatacagcaaaagagtccttaagggtcaaaattattcatttaaatacaaaaataa
   N N D T A K S L K V Q N Y S F K Y K N N
301 cccatttttaggattttgcaggagctattgggttattcaataggcaattcaagaatagaact
   P F L G F A G A I G Y S I G N S R I E L
361 agaagtatcacatgaaatatttgatactaaaaaccaggaaacaattatttaattgactc
   E V S H E I F D T K N P G N N Y L N D S
421 tcacaaatattgcgcttttatctcatggaagtacatatgcagtgatggaaatagcggaga
   H K Y C A L S H G S H I C S D G N S G D
481 ttggtacactgcaaaaactgataagtttgactttctgaaaaactgaagggtttacttgacgt
   W Y T A K T D K P V L L K N E G L L D V
541 ctcattttatggttaaacgcgtgttatgacataacaactgaaaaaatgcctttttcacctta
   S F M L N A C Y D I T T E K M P F S P Y
601 tatatgtgcagggtattgggtactgatctcatatcttatgtttgagacacacaaaaacaaat
   I C A G I G T D L I S M F E T T Q N K I
661 atctttatcaaggaaagttagggttttaactataactataaaactcaagagttctgtttttgc
   S Y Q G K L G L N Y T I N S R V S V F A
721 aggtgggcacttttcataaggtaataggtaatgaatttaagggtattcttactctattacc
   G G H F H K V I G N E F K G I P T L L P
781 tgatggatcaaacattaaagtacaacagtcctgcaacagtaacattagatgtgtgcccattt
   D G S N I K V Q Q S A T V T L D V C H F
841 cgggttagagattggaagtagatttttttttaataactttctattgtacatgttaaaaaata
   G L E I G S R F F F
901 gtacttagtttgccttctgtggtttataaaacgcaagagagaaatagttagtaataaattaga
961 aagttaaaattagaaaaagtcatatgtttttcattgctattgatactcaactaaaaagtag
1021 tataaatgttactttattaataattttacgtagtatattaaatttcccttacaaaagccac
1081 tagtatttttatactaaaagcttatactttgggttgatttttaatttgtatttttactactgt
   -35      -10
1141 taattttactttcactgctttttggtgtaaatatgaattgtaaaaaagttttcacataaagt
   RBS      M N C K K V F T I S
1201 gcattgatatcatccatatacttctacctaattgtctcatatcttaaccagttataggt
   A L I S S I Y F L P N V S Y S N P V Y G
1261 aacagttatgtatggtaatttttcatatcaggaaggtacatgccaagtgttctctatttt
   N S M Y G N F Y I S G K Y M P S V P H F
1321 ggaattttttcagctgaagaagagaaaaaaagacaactgtagtatatggcttaaaagaa
   G I F S A E E E K K K T T V V Y G L K E
1381 aactgggcaggagatgcaatatctagtcaaagtcagatgataattttaccatttcgaaat
   N W A G D A I S S Q S P D D N F T I R N
1441 tactcatccaagtatgcaagcaacaagtttttaggggtttgcagtagctattgggtactcg
   Y S F K Y A S N K F L G F A V A I G Y S
1501 ataggcagtcgaagaatagaagttgagatgtcttatgaagcatttgatgtaaaaaatcaa
   I G S P R I E V E M S Y E A P D V K N Q
1561 ggtaacaatt
      G N N

```

FIG. 2C

1 acatgtatacattatagtaacaaatgttacggtatTTTTattcataagttaagtaaaatct  
 61 ataccattctcttttcactttatcagaagactttttatttatcaciaaactcatgacgtatag  
 121 tgtcaciaaataaacacactgcaactgcaatcactacgtaaaactttaactcttcttttttc  
 181 acaactaaaatactaataaaaagtaatatagtataaaaaaatcttaagtaacTTGACAtaat  
 -35  
 241 attactctgataTAGCATatgtctagtatctctataactaaacgtttatataattGGAGCca  
 -10  
 301 tattaATGAAAGCTATCAAATTCATACTTAATGTCTGCTTACTATTTGCAGCAATATTTT  
 M K A I K F I L N V C L L F A → A I F L  
 361 TAGGGTATTCCTATATTACAAAACAAGGCATATTTCAAACAAAACATCATGATACACCTA  
 G Y S Y I T K Q G I F Q T K H H D T P N  
 421 ATACTACTATACCAAATGAAGACGGTATTCAATCTAGCTTTAGCTTAATCAATCAAGACG  
 T T I P N E D G I Q S S F S L I N Q D G  
 481 GTAAAACAGTAACCAGCCAAGATTTCTAGGGAAACACATGTTAGTTTTGTTTGGATTCT  
 K T V T S Q D F L G K H M L V L F G F S  
 541 CTGCATGTAAAAGCATTTGCCCTGCAGAATTGGGATTAGTATCTGAAGCACTTGCACAAC  
 A C K S I C P A E L G L V S E A L A Q L  
 601 TTGGTAATAATGCAGACAAATTACAAGTAATTTTTATTACAATTGATCCAAAAAATGATA  
 G N N A D K L Q V I F I T I D P K N D T  
 661 CTGTAGAAAAATTAAAAGAATTTTCATGAACATTTTGATTCAAGAATTCAAATGTTAACAG  
 V E K L K E F H E H F D S R I Q M L T G  
 721 GAAATACTGAAGACATTAATCAAATAATTTAAAATTTATAAAATATATGTTGGACAAGCAG  
 N T E D I N Q I I K N Y K I Y V G Q A D  
 781 ATAAAGATCATCAAATTAACCATTTCTGCAATAATGTACCTTATTGACAAAAAAGGATCAT  
 K D H Q I N H S A I M Y L I D K K G S Y  
 841 ATCTTTCACACTTCATTCCAGATTTAAAATCACAAGAAAATCAAGTAGATAAGTTACTAT  
 L S H F I P D L K S Q E N Q V D K L L S  
 901 CTTTAGTTAAGCAGTATCTGTAAtttaataattaattAAAGagaatagtagacacCTTTtt  
 L V K Q Y L \*  
 961 ataaattcatggaatacgttggtgagtaggttttttttagtatTTTTtagtgctaataac  
 1021 attggcat

FIG. 3A

1 ggaaatctcatgtaaacgtgaaatactatattcttttttaaataccaatacaattgaata  
 61 caaaaaaaccttttacaacttattatgtttatcttaaaaccttattttaagattccttatg  
 121 tcacaaaataacaaaaatactattttacaaaatacaccacaatttcatcaaataaaaaaaaa  
 181 ctatacactttattatactacagtagatataccataaaagattttaagtaacTTGACAta  
 241 atattaccttggtatTAGCATatgattcagtatttttatattaaaattttattatgtattGGA  
 -35  
 301 GcataaaaATGAAAGTTATCAAATTTATACTTAATATCTGTTTATTATTTGCAGCAATTTT  
 -10  
 M K V I K F I L N I C L L F A → A I F  
 361 TCTAGGATATTCCCTACGTAACAAAACAAGGCATTTTCAAGTAAGAGATCATAACACTCC  
 L G Y S Y V T K Q G I F Q V R D H N T P  
 421 CAATACAAATATATCAAATAAAGCCAGCATTACTACTAGTTTTTCGTTAGTAAATCAAGA  
 N T N I S N K A S I T T S F S L V N Q D  
 481 TGGAAATACAGTAAATAGTCAAGATTTTTTGGGAAAATACATGCTAGTTTTATTGGATT  
 G N T V N S Q D F L G K Y M L V L F G F  
 541 TTCTTCATGTAAAAGCATCTGCCCTGCTGAATTAGGAATAGCATCTGAAGTTCTCTCACA  
 S S C K S I C P A E L G I A S E V L S Q  
 601 GCTTGGTAATGACACAGACAAGTTACAAGTAATTTTCATTACAATTGATCCAACAAATGA  
 L G N D T D K L Q V I F I T I D P T N D  
 661 TACTGTACAAAAATTAAAAACATTTTCATGAACATTTTGATCCTAGAATTCAAATGCTAAC  
 T V Q K L K T F H E H F D P R I Q M L T  
 721 AGGCAGTGCAGAAGATATTGAAAAAATAATAAAAAATTACAAAATATATGTTGGACAAGC  
 G S A E D I E K I I K N Y K I Y V G Q A  
 781 AGATAAAGATAATCAAATTGATCACTCTGCCATAATGTACATTATCGATAAAAAAGGAGA  
 D K D N Q I D H S A I M Y I I D K K G E  
 841 ATACATTTCACACTTTTCTCCAGATTTAAATCAACAGAAAATCAAGTAGATAAGTTACT  
 Y I S H F S P D L K S T E N Q V D K L L  
 901 ATCTATAATAAAACAATATCTCTAAtttaataattaattaAAGAGaatagtagacacaCTCT  
 S I I K Q Y L \*  
 961 Tatataaattcatggatatatgtgatgggtagatttcttttggtggttctatcgctaatt  
 1021 acatta

FIG. 3B

## SEQUENCE LISTING

<110> University of Florida

<120> Nucleic Acid Vaccines Against Rickettsial Diseases and  
Methods of use

<130> UF-167C3

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<150> 08/953,326

<151> 1997-10-17

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tca ttt tta cct ggt gtg tcc ttt tct gat gta ata cag gaa gac agc 96  
Ser Phe Leu Pro Gly Val Ser Phe Ser Asp Val Ile Gln Glu Asp Ser  
20 25 30

aac cca gca ggc agt gtt tac att agc gca aaa tac atg cca act gca 144  
Asn Pro Ala Gly Ser Val Tyr Ile Ser Ala Lys Tyr Met Pro Thr Ala  
35 40 45

tca cat ttt ggt aaa atg tca atc aaa gaa gat tca aaa aat act caa 192  
Ser His Phe Gly Lys Met Ser Ile Lys Glu Asp Ser Lys Asn Thr Gln  
50 55 60

acg gta ttt ggt cta aaa aaa gat tgg gat ggc gtt aaa aca cca tca 240  
Thr Val Phe Gly Leu Lys Lys Asp Trp Asp Gly Val Lys Thr Pro Ser  
65 70 75 80

gat tct agc aat act aat tct aca att ttt act gaa aaa gac tat tct 288

2

Asp	Ser	Ser	Asn	Thr	Asn	Ser	Thr	Ile	Phe	Thr	Glu	Lys	Asp	Tyr	Ser		
				85					90					95			
ttc	aga	tat	gaa	aac	aat	ccg	ttt	tta	ggt	ttc	gct	gga	gca	att	ggg	336	
Phe	Arg	Tyr	Glu	Asn	Asn	Pro	Phe	Leu	Gly	Phe	Ala	Gly	Ala	Ile	Gly		
			100					105					110				
tac	tca	atg	aat	gga	cca	aga	ata	gag	ttc	gaa	gta	tcg	tat	gaa	act	384	
Tyr	Ser	Met	Asn	Gly	Pro	Arg	Ile	Glu	Phe	Glu	Val	Ser	Tyr	Glu	Thr		
			115					120					125				
ttt	gat	gta	aaa	aac	cta	ggt	ggc	aac	tat	aaa	aac	aac	gca	cac	atg	432	
Phe	Asp	Val	Lys	Asn	Leu	Gly	Gly	Asn	Tyr	Lys	Asn	Asn	Ala	His	Met		
			130					135					140				
tac	tgt	gct	tta	gat	aca	gca	gca	caa	aat	agc	act	aat	ggc	gca	gga	480	
Tyr	Cys	Ala	Leu	Asp	Thr	Ala	Ala	Gln	Asn	Ser	Thr	Asn	Gly	Ala	Gly		
						150				155					160		
tta	act	aca	tct	gtt	atg	gta	aaa	aac	gaa	aat	tta	aca	aat	ata	tca	528	
Leu	Thr	Thr	Ser	Val	Met	Val	Lys	Asn	Glu	Asn	Leu	Thr	Asn	Ile	Ser		
				165					170					175			
tta	atg	tta	aat	gcg	tgt	tat	gat	atc	atg	ctt	gat	gga	ata	cca	gtt	576	
Leu	Met	Leu	Asn	Ala	Cys	Tyr	Asp	Ile	Met	Leu	Asp	Gly	Ile	Pro	Val		
				180				185					190				
tct	cca	tat	gta	tgt	gca	ggt	att	ggc	act	gac	tta	gtg	tca	gta	att	624	
Ser	Pro	Tyr	Val	Cys	Ala	Gly	Ile	Gly	Thr	Asp	Leu	Val	Ser	Val	Ile		
			195					200					205				
aat	gct	aca	aat	cct	aaa	tta	tct	tat	caa	gga	aag	cta	ggc	ata	agt	672	
Asn	Ala	Thr	Asn	Pro	Lys	Leu	Ser	Tyr	Gln	Gly	Lys	Leu	Gly	Ile	Ser		
			210					215					220				
tac	tca	atc	aat	tct	gaa	gct	tct	atc	ttt	atc	ggt	gga	cat	ttc	cat	720	
Tyr	Ser	Ile	Asn	Ser	Glu	Ala	Ser	Ile	Phe	Ile	Gly	Gly	His	Phe	His		
					230				235					240			
aga	gtt	ata	ggt	aat	gaa	ttt	aaa	gat	att	gct	acc	tta	aaa	ata	ttt	768	
Arg	Val	Ile	Gly	Asn	Glu	Phe	Lys	Asp	Ile	Ala	Thr	Leu	Lys	Ile	Phe		
				245					250					255			
act	tca	aaa	aca	gga	ata	tct	aat	cct	ggc	ttt	gca	tca	gca	aca	ctt	816	
Thr	Ser	Lys	Thr	Gly	Ile	Ser	Asn	Pro	Gly	Phe	Ala	Ser	Ala	Thr	Leu		
				260				265					270				
gat	gtt	tgt	cac	ttt	ggt	ata	gaa	att	gga	gga	agg	ttt	gta	ttt	taa	864	
Asp	Val	Cys	His	Phe	Gly	Ile	Glu	Ile	Gly	Gly	Arg	Phe	Val	Phe			
				275				280					285				

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&lt;212&gt; PRT

&lt;213&gt; Cowdria ruminantium

&lt;400&gt; 2

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Ser Phe Leu Pro Gly Val Ser Phe Ser Asp Val Ile Gln Glu Asp Ser  
 20 25 30

Asn Pro Ala Gly Ser Val Tyr Ile Ser Ala Lys Tyr Met Pro Thr Ala  
 35 40 45

Ser His Phe Gly Lys Met Ser Ile Lys Glu Asp Ser Lys Asn Thr Gln  
 50 55 60

Thr Val Phe Gly Leu Lys Lys Asp Trp Asp Gly Val Lys Thr Pro Ser  
 65 70 75 80

Asp Ser Ser Asn Thr Asn Ser Thr Ile Phe Thr Glu Lys Asp Tyr Ser  
 85 90 95

Phe Arg Tyr Glu Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly  
 100 105 110

Tyr Ser Met Asn Gly Pro Arg Ile Glu Phe Glu Val Ser Tyr Glu Thr  
 115 120 125

Phe Asp Val Lys Asn Leu Gly Gly Asn Tyr Lys Asn Asn Ala His Met  
 130 135 140

Tyr Cys Ala Leu Asp Thr Ala Ala Gln Asn Ser Thr Asn Gly Ala Gly  
 145 150 155 160

Leu Thr Thr Ser Val Met Val Lys Asn Glu Asn Leu Thr Asn Ile Ser  
 165 170 175

Leu Met Leu Asn Ala Cys Tyr Asp Ile Met Leu Asp Gly Ile Pro Val  
 180 185 190

Ser Pro Tyr Val Cys Ala Gly Ile Gly Thr Asp Leu Val Ser Val Ile  
 195 200 205

Asn Ala Thr Asn Pro Lys Leu Ser Tyr Gln Gly Lys Leu Gly Ile Ser  
 210 215 220

Tyr Ser Ile Asn Ser Glu Ala Ser Ile Phe Ile Gly Gly His Phe His  
 225 230 235 240

Arg Val Ile Gly Asn Glu Phe Lys Asp Ile Ala Thr Leu Lys Ile Phe  
 245 250 255

Thr Ser Lys Thr Gly Ile Ser Asn Pro Gly Phe Ala Ser Ala Thr Leu  
 260 265 270

Asp Val Cys His Phe Gly Ile Glu Ile Gly Gly Arg Phe Val Phe

275

280

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   1                  5                  10                  15

ctt ctc tta cct gga gta tca ttt tcc gac cca agg cag gta gtg gtc 96  
 Leu Leu Leu Pro Gly Val Ser Phe Ser Asp Pro Arg Gln Val Val Val  
                   20                  25                  30

att aac ggt aat ttc tac atc agt gga aaa tac gat gcc aag gct tcg 144  
 Ile Asn Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Asp Ala Lys Ala Ser  
                   35                  40                  45

cat ttt gga gta ttc tct gct aag gaa gaa aga aat aca aca gtt gga 192  
 His Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly  
                   50                  55                  60

gtg ttt gga ctg aag caa aat tgg gac gga agc gca ata tcc aac tcc 240  
 Val Phe Gly Leu Lys Gln Asn Trp Asp Gly Ser Ala Ile Ser Asn Ser  
                   65                  70                  75                  80

tcc cca aac gat gta ttc act gtc tca aat tat tca ttt aaa tat gaa 288  
 Ser Pro Asn Asp Val Phe Thr Val Ser Asn Tyr Ser Phe Lys Tyr Glu  
                   85                  90                  95

aac aac ccg ttt tta ggt ttt gca gga gct att ggt tac tca atg gat 336  
 Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp  
                   100                  105                  110

ggt cca aga ata gag ctt gaa gta tct tat gaa aca ttt gat gta aaa 384  
 Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Thr Phe Asp Val Lys  
                   115                  120                  125

aat caa ggt aac aat tat aag aat gaa gca cat aga tat tgt gct cta 432  
 Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Cys Ala Leu  
                   130                  135                  140

tcc cat aac tca gca gca gac atg agt agt gca agt aat aat ttt gtc 480  
 Ser His Asn Ser Ala Ala Asp Met Ser Ser Ala Ser Asn Asn Phe Val  
                   145                  150                  155                  160

ttt cta aaa aat gaa gga tta ctt gac ata tca ttt atg ctg aac gca 528  
 Phe Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Phe Met Leu Asn Ala

	165	170	175	
tgc tat gac gta gta ggc gaa ggc ata cct ttt tct cct tat ata tgc				576
Cys Tyr Asp Val Val Gly Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys				
	180	185	190	
gca ggt atc ggt act gat tta gta tcc atg ttt gaa gct aca aat cct				624
Ala Gly Ile Gly Thr Asp Leu Val Ser Met Phe Glu Ala Thr Asn Pro				
	195	200	205	
aaa att tct tac caa gga aag tta ggt tta agc tac tct ata agc cca				672
Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro				
	210	215	220	
gaa gct tct gtg ttt att ggt ggg cac ttt cat aag gta ata ggg aac				720
Glu Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn				
	225	230	235	240
gaa ttt aga gat att cct act ata ata cct act gga tca aca ctt gca				768
Glu Phe Arg Asp Ile Pro Thr Ile Ile Pro Thr Gly Ser Thr Leu Ala				
	245	250	255	
gga aaa gga aac tac cct gca ata gta ata ctg gat gta tgc cac ttt				816
Gly Lys Gly Asn Tyr Pro Ala Ile Val Ile Leu Asp Val Cys His Phe				
	260	265	270	
gga ata gaa atg gga gga agg ttt aa				842
Gly Ile Glu Met Gly Gly Arg Phe				
	275	280		

&lt;210&gt; 4

&lt;211&gt; 280

&lt;212&gt; PRT

&lt;213&gt; Ehrlichia chaffeensis

&lt;400&gt; 4

Met Asn Tyr Lys Lys Ser Phe Ile Thr Ala Ile Asp Ile Ile Asn Ile
1 5 10 15

Leu Leu Leu Pro Gly Val Ser Phe Ser Asp Pro Arg Gln Val Val Val
20 25 30

Ile Asn Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Asp Ala Lys Ala Ser
35 40 45

His Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly
50 55 60

Val Phe Gly Leu Lys Gln Asn Trp Asp Gly Ser Ala Ile Ser Asn Ser
65 70 75 80

Ser Pro Asn Asp Val Phe Thr Val Ser Asn Tyr Ser Phe Lys Tyr Glu
85 90 95

Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp  
                   100                  105                  110  
 Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Thr Phe Asp Val Lys  
                   115                  120                  125  
 Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Cys Ala Leu  
                   130                  135                  140  
 Ser His Asn Ser Ala Ala Asp Met Ser Ser Ala Ser Asn Asn Phe Val  
                   145                  150                  155                  160  
 Phe Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Phe Met Leu Asn Ala  
                   165                  170                  175  
 Cys Tyr Asp Val Val Gly Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys  
                   180                  185                  190  
 Ala Gly Ile Gly Thr Asp Leu Val Ser Met Phe Glu Ala Thr Asn Pro  
                   195                  200                  205  
 Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro  
                   210                  215                  220  
 Glu Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn  
                   225                  230                  235                  240  
 Glu Phe Arg Asp Ile Pro Thr Ile Ile Pro Thr Gly Ser Thr Leu Ala  
                   245                  250                  255  
 Gly Lys Gly Asn Tyr Pro Ala Ile Val Ile Leu Asp Val Cys His Phe  
                   260                  265                  270  
 Gly Ile Glu Met Gly Gly Arg Phe  
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tgc gcc tgc tcc cta ctt gtt agt ggg gcc gta gtg gca tct ccc atg	96
Cys Ala Cys Ser Leu Leu Val Ser Gly Ala Val Val Ala Ser Pro Met	
20 25 30	
agt cac gaa gtg gct tct gaa ggg gga gta atg gga ggt agc ttt tac	144
Ser His Glu Val Ala Ser Glu Gly Gly Val Met Gly Gly Ser Phe Tyr	
35 40 45	
gtg ggt gcg gcc tac agc cca gca ttt cct tct gtt acc tcg ttc gac	192
Val Gly Ala Ala Tyr Ser Pro Ala Phe Pro Ser Val Thr Ser Phe Asp	
50 55 60	
atg cgt gag tca agc aaa gag acc tca tac gtt aga ggc tat gac aag	240
Met Arg Glu Ser Ser Lys Glu Thr Ser Tyr Val Arg Gly Tyr Asp Lys	
65 70 75 80	
agc att gca acg att gat gtg agt gtg cca gca aac ttt tcc aaa tct	288
Ser Ile Ala Thr Ile Asp Val Ser Val Pro Ala Asn Phe Ser Lys Ser	
85 90 95	
ggc tac act ttt gcc ttc tct aaa aac tta atc acg tct ttc gac ggc	336
Gly Tyr Thr Phe Ala Phe Ser Lys Asn Leu Ile Thr Ser Phe Asp Gly	
100 105 110	
gct gtg gga tat tct ctg gga gga gcc aga gtg gaa ttg gaa gcg agc	384
Ala Val Gly Tyr Ser Leu Gly Gly Ala Arg Val Glu Leu Glu Ala Ser	
115 120 125	
tac aga agg ttt gct act ttg gcg gac ggg cag tac gca aaa agt ggt	432
Tyr Arg Arg Phe Ala Thr Leu Ala Asp Gly Gln Tyr Ala Lys Ser Gly	
130 135 140	
gcg gaa tct ctg gca gct att acc cgc gac gct aac att act gag acc	480
Ala Glu Ser Leu Ala Ala Ile Thr Arg Asp Ala Asn Ile Thr Glu Thr	
145 150 155 160	
aat tac ttc gta gtc aaa att gat gaa atc aca aac acc tca gtc atg	528
Asn Tyr Phe Val Val Lys Ile Asp Glu Ile Thr Asn Thr Ser Val Met	
165 170 175	
tta aat ggc tgc tat gac gtg ctg cac aca gat tta cct gtg tcc ccg	576
Leu Asn Gly Cys Tyr Asp Val Leu His Thr Asp Leu Pro Val Ser Pro	
180 185 190	
tat gta tgt gcc ggg ata ggc gca agc ttt gtt gac atc tct aag caa	624
Tyr Val Cys Ala Gly Ile Gly Ala Ser Phe Val Asp Ile Ser Lys Gln	
195 200 205	
gta acc aca aag ctg gcc tac agg ggc aag gtt ggg att agc tac cag	672
Val Thr Thr Lys Leu Ala Tyr Arg Gly Lys Val Gly Ile Ser Tyr Gln	
210 215 220	

ttt act ccg gaa ata tcc ttg gtg gca ggt ggg ttc tac cac ggg cta 720  
 Phe Thr Pro Glu Ile Ser Leu Val Ala Gly Gly Phe Tyr His Gly Leu  
 225 230 235 240

ttt gat gag tct tac aag gac att ccc gca cac aac agt gta aag ttc 768  
 Phe Asp Glu Ser Tyr Lys Asp Ile Pro Ala His Asn Ser Val Lys Phe  
 245 250 255

tct gga gaa gca aaa gcc tca gtc aaa gcg cat att gct gac tac ggc 816  
 Ser Gly Glu Ala Lys Ala Ser Val Lys Ala His Ile Ala Asp Tyr Gly  
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 35 40 45

Val Gly Ala Ala Tyr Ser Pro Ala Phe Pro Ser Val Thr Ser Phe Asp  
 50 55 60

Met Arg Glu Ser Ser Lys Glu Thr Ser Tyr Val Arg Gly Tyr Asp Lys  
 65 70 75 80

Ser Ile Ala Thr Ile Asp Val Ser Val Pro Ala Asn Phe Ser Lys Ser  
 85 90 95

Gly Tyr Thr Phe Ala Phe Ser Lys Asn Leu Ile Thr Ser Phe Asp Gly  
 100 105 110

Ala Val Gly Tyr Ser Leu Gly Gly Ala Arg Val Glu Leu Glu Ala Ser  
 115 120 125

Tyr Arg Arg Phe Ala Thr Leu Ala Asp Gly Gln Tyr Ala Lys Ser Gly  
 130 135 140

Ala Glu Ser Leu Ala Ala Ile Thr Arg Asp Ala Asn Ile Thr Glu Thr  
 145 150 155 160

Asn Tyr Phe Val Val Lys Ile Asp Glu Ile Thr Asn Thr Ser Val Met  
 165 170 175

Leu Asn Gly Cys Tyr Asp Val Leu His Thr Asp Leu Pro Val Ser Pro  
                   180                                  185                                  190  
 Tyr Val Cys Ala Gly Ile Gly Ala Ser Phe Val Asp Ile Ser Lys Gln  
                   195                                  200                                  205  
 Val Thr Thr Lys Leu Ala Tyr Arg Gly Lys Val Gly Ile Ser Tyr Gln  
                   210                                  215                                  220  
 Phe Thr Pro Glu Ile Ser Leu Val Ala Gly Gly Phe Tyr His Gly Leu  
                   225                                  230                                  235                                  240  
 Phe Asp Glu Ser Tyr Lys Asp Ile Pro Ala His Asn Ser Val Lys Phe  
                                   245                                  250                                  255  
 Ser Gly Glu Ala Lys Ala Ser Val Lys Ala His Ile Ala Asp Tyr Gly  
                                   260                                  265                                  270  
 Phe Asn Leu Gly Ala Arg Phe Leu Phe Ser  
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 ctccagactt agcaacagta acactgagtg tgtgtcactt tggagtagaa cttggaggaa 120  
 gatttaactt ct 132

<210> 8  
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 ctggaatata accttctgat ccagtacagg atgacaacat tagtggtaat ttctacatca 120  
 gtggaaagta tatgccaagc gcttcgcatt ttggagtttt ttctgccaaag gaagaaagaa 180  
 atacaacagt tggagtattt ggaatagagc aagattggga tagatgtgta atatctagaa 240  
 ccactttaag cgatatatcc accgttccaa attattcatt taagtatgaa aataatctat 300  
 tttcaggatt tgcaggagct attggctact caatggatgg cccaagaata gagcttgaag 360  
 tatcttatga agcattcgat gttaaaaatc aaggtaacaa ttataagaac gaagcacata 420

gatattatgc tctgtcccat cttctcggca cagagacaca gatagatggt gcaggcagtg 480  
cgtctgtctt tctaataaat gaaggactac ttgataaatc atttatgctg aacgcatggt 540  
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taagttaccc tataagccca gaagcttctg tgtttattgg tggacatttt cataaggtga 720  
taggaaacga atntagagat attcctacta tgatacctag tgaatcagcg cttgcaggaa 780  
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gaaggtttaa cttccaactt t 861

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<213> Ehrlichia chaffeensis

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gtggcaagta tatgccagt gcttcgcatt ttggcatggt ttctgccaaa gaagaaaaaa 180  
atcctactgt tgcattgtat ggcttaaaac aagattggga agggattagc tcatcaagtc 240  
acaatgataa tcatttcaat aacaagggtt attcatttaa atatgaaaat aaccattttt 300  
tagggtttgc aggagctatt ggttattcaa tgggtggtcc aagagtagag tttgaagtgt 360  
cctatgaaac atttgacgtt aaaaatcagg gtaataacta taaaatgat gctcacagat 420  
actgtgcttt aggtcaacaa gacaacagcg gaatacctaa aactagtaaa tacgtactgt 480  
taaaaagcga aggattgctt gacatatcat ttatgctaaa tgcattgctat gatataataa 540  
acgagagcat acctttgtct cttacatat gtgcagggtg tggtagtgat ttaatatcca 600  
tgtttgaagc tacaaatcct aaaatttctt accaaggga gttaggtcta agttactcta 660  
taaaccaga agcttctgta ttattgggtg gacattttca taagggtgata ggaaacgaat 720  
ttagggacat tctactctg aaagcatttg ttacgtcacc agctactcca gatctagcaa 780  
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<210> 10  
<211> 843  
<212> DNA

<213> Ehrlichia chaffeensis

<400> 10

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attctccaga aaatacattt aacgttccaa attattcatt taaatatgaa aataatccat 300
ttctaggttt tgcaggagct gttggttatt taatgaatgg tccaagaata gagttagaaa 360
tgtcctatga aacatttgat gtgaaaaacc agggtaataa ctataagaac gatgctcaca 420
aatattatgc ttttaacccat aacagtgggg gaaagctaag caatgcaggt gataagtttg 480
tttttctaaa aaatgaagga ctacttgata tatcacttat gttgaatgca tgctatgatg 540
taataagtga aggaatacct ttctctcctt acatatgtgc aggtgttggt actgatttaa 600
tatccatggt tgaagctata aaccctaaaa tttcttatca aggaaagtta ggtttgagtt 660
actccataag cccagaagct tctgtttttg ttgggtggaca ttttcataag gtgataggga 720
atgaattcag agatattcct gctatgatac ccagtacctc aactctcaca ggtaatcact 780
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843

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<210> 11

<211> 830

<212> DNA

<213> Ehrlichia chaffeensis

<400> 11

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gaaaatacat gccaaagtgc tcgcattttg gagtattctc tgctaaggaa gaaagaaata 180
caacagttgg agtgtttgga ctgaagcaaa attgggacgg aagcgcaata tocaactcct 240
cccccacga tgtattcact gtctcaaatt attcatttaa atatgaaaac aaccogtttt 300
taggttttgc aggagctatt ggttactcaa tggatggtcc aagaatagag cttgaagtat 360
cttatgaaac atttgatgta aaaaatcaag gtaacaatta taagaatgaa gcacatagat 420
attgtgctct atcccataac tcagcagcag acatgagtag tgcaagtaat aattttgtct 480

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ttctaaaaaa tgaaggatta cttgacatat catttatgct gaacgcatgc tatgacgtag 540  
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 ccatgtttga agctacaaat cctaaaattt cttaccaagg aaagttaggt ttaagctact 660  
 ctataagccc agaagcttct gtgtttattg gtgggcactt tcataaggta atagggaacg 720  
 aatttagaga tattcctact ataataccta ctggatcaac acttgcagga aaaggaaact 780  
 accctgcaat agtaatactg gatgtatgcc actttggaat agaaatggga 830

&lt;210&gt; 12

&lt;211&gt; 864

&lt;212&gt; DNA

&lt;213&gt; Ehrlichia canis

&lt;400&gt; 12

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 gaaaatatat gccaacagcg tcacattttg gaattttttc agctaaagaa gaacaaagtt 180  
 ttactaaggt attagttggg ttagatcaac gattatcaca taatattata aacaataatg 240  
 atacagcaaa gagtcttaag gttcaaaatt attcatttaa atacaaaaat aaccatttc 300  
 taggatttgc aggagctatt ggttattcaa taggcaattc aagaatagaa ctagaagtat 360  
 cacatgaaat atttgatact aaaaaccag gaaacaatta tttaaagac tctcacaaat 420  
 attgcgcttt atctcatgga agtcacatat gcagtgatgg aaatagcgga gattggtaca 480  
 ctgcaaaaac tgataagttt gtacttctga aaaatgaagg tttacttgac gtctcattta 540  
 tgttaaacgc atgttatgac ataacaactg aaaaaatgcc tttttcacct tatatatgtg 600  
 caggtattgg tactgatctc atatctatgt ttgagacaac aaaaaacaaa atatcttatt 660  
 aaggaaagtt aggtttaaac tatactataa actcaagagt ttctgttttt gcaggtgggc 720  
 actttcataa ggtaataggt aatgaattta aaggatttcc tactctatta cctgatggat 780  
 caaacattaa agtacaacag tctgcaacag taacattaga tgtgtgccat ttcgggttag 840  
 agattggaag tagatttttc tttt 864

&lt;210&gt; 13

&lt;211&gt; 399

&lt;212&gt; DNA

&lt;213&gt; Ehrlichia canis

13

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 caggaaagta catgccaaagt gttcctcatt ttggaatttt ttcagctgaa gaagagaaaa 180  
 aaaagacaac tgtagtatat ggcttaaaaag aaaactgggc aggagatgca atatctagtc 240  
 aaagtccaga tgataatttt accattcgaa attactcatt caagtatgca agcaacaagt 300  
 ttttaggggtt tgcagtagct attgggttact cgataggcag tccaagaata gaagttgaga 360  
 tgtcttatga agcatttgat gtaaaaaatc aaggttaaca 399

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 <211> 43  
 <212> PRT  
 <213> Ehrlichia chaffeensis

<400> 14  
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 <211> 286  
 <212> PRT  
 <213> Ehrlichia chaffeensis

<400> 15  
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 20 25 30  
 Ile Ser Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Ala Ser  
 35 40 45  
 His Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly  
 50 55 60  
 Val Phe Gly Ile Glu Gln Asp Trp Asp Arg Cys Val Ile Ser Arg Thr  
 65 70 75 80  
 Thr Leu Ser Asp Ile Phe Thr Val Pro Asn Tyr Ser Phe Lys Tyr Glu  
 85 90 95

14

Asn Asn Leu Phe Ser Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp  
 100 105 110  
 Gly Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Ala Phe Asp Val Lys  
 115 120 125  
 Asn Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Tyr Ala Leu  
 130 135 140  
 Ser His Leu Leu Gly Thr Glu Thr Gln Ile Asp Gly Ala Gly Ser Ala  
 145 150 155 160  
 Ser Val Phe Leu Ile Asn Glu Gly Leu Leu Asp Lys Ser Phe Met Leu  
 165 170 175  
 Asn Ala Cys Tyr Asp Val Ile Ser Glu Gly Ile Pro Phe Ser Pro Tyr  
 180 185 190  
 Ile Cys Ala Gly Ile Gly Ile Asp Leu Val Ser Met Phe Glu Ala Ile  
 195 200 205  
 Asn Pro Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Pro Ile  
 210 215 220  
 Ser Pro Glu Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile  
 225 230 235 240  
 Gly Asn Glu Phe Arg Asp Ile Pro Thr Met Ile Pro Ser Glu Ser Ala  
 245 250 255  
 Leu Ala Gly Lys Gly Asn Tyr Pro Ala Ile Val Thr Leu Asp Val Phe  
 260 265 270  
 Tyr Phe Gly Ile Glu Leu Gly Gly Arg Phe Asn Phe Gln Leu  
 275 280 285

<210> 16  
 <211> 278  
 <212> PRT  
 <213> Ehrlichia chaffeensis

<400> 16  
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 20 25 30  
 Ile Ser Gly Asn Phe Tyr Val Ser Gly Lys Tyr Met Pro Ser Ala Ser  
 35 40 45  
 His Phe Gly Met Phe Ser Ala Lys Glu Glu Lys Asn Pro Thr Val Ala  
 50 55 60

15

Leu Tyr Gly Leu Lys Gln Asp Trp Glu Gly Ile Ser Ser Ser Ser His  
 65 70 75 80  
 Asn Asp Asn His Phe Asn Asn Lys Gly Tyr Ser Phe Lys Tyr Glu Asn  
 85 90 95  
 Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Gly Gly  
 100 105 110  
 Pro Arg Val Glu Phe Glu Val Ser Tyr Glu Thr Phe Asp Val Lys Asn  
 115 120 125  
 Gln Gly Asn Asn Tyr Lys Asn Asp Ala His Arg Tyr Cys Ala Leu Gly  
 130 135 140  
 Gln Gln Asp Asn Ser Gly Ile Pro Lys Thr Ser Lys Tyr Val Leu Leu  
 145 150 155 160  
 Lys Ser Glu Gly Leu Leu Asp Ile Ser Phe Met Leu Asn Ala Cys Tyr  
 165 170 175  
 Asp Ile Ile Asn Glu Ser Ile Pro Leu Ser Pro Tyr Ile Cys Ala Gly  
 180 185 190  
 Val Gly Thr Asp Leu Ile Ser Met Phe Glu Ala Thr Asn Pro Lys Ile  
 195 200 205  
 Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Asn Pro Glu Ala  
 210 215 220  
 Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn Glu Phe  
 225 230 235 240  
 Arg Asp Ile Pro Thr Leu Lys Ala Phe Val Thr Ser Ser Ala Thr Pro  
 245 250 255  
 Asp Leu Ala Ile Val Thr Leu Ser Val Cys His Phe Gly Ile Glu Leu  
 260 265 270  
 Gly Gly Arg Phe Asn Phe  
 275

&lt;210&gt; 17

&lt;211&gt; 280

&lt;212&gt; PRT

&lt;213&gt; Ehrlichia chaffeensis

&lt;400&gt; 17

Met Asn Cys Lys Lys Phe Phe Ile Thr Thr Thr Leu Val Ser Leu Met  
 1 5 10 15  
 Ser Phe Leu Pro Gly Ile Ser Phe Ser Asp Ala Val Gln Asn Asp Asn  
 20 25 30

16

Val Gly Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Val Pro Ser Val Ser  
                   35                                  40                                  45  
 His Phe Gly Val Phe Ser Ala Lys Gln Glu Arg Asn Thr Thr Ile Gly  
                   50                                  55                                  60  
 Val Phe Gly Leu Lys Gln Asp Trp Asp Gly Ser Thr Ile Ser Lys Asn  
                   65                                  70                                  75                                  80  
 Ser Pro Glu Asn Thr Phe Asn Val Pro Asn Tyr Ser Phe Lys Tyr Glu  
                                   85                                  90                                  95  
 Asn Asn Pro Phe Leu Gly Phe Ala Gly Ala Val Gly Tyr Leu Met Asn  
                                   100                                  105                                  110  
 Gly Pro Arg Ile Glu Leu Glu Met Ser Tyr Glu Thr Phe Asp Val Lys  
                                   115                                  120                                  125  
 Asn Gln Gly Asn Asn Tyr Lys Asn Asp Ala His Lys Tyr Tyr Ala Leu  
                                   130                                  135                                  140  
 Thr His Asn Ser Gly Gly Lys Leu Ser Asn Ala Gly Asp Lys Phe Val  
                                   145                                  150                                  155                                  160  
 Phe Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Leu Met Leu Asn Ala  
                                   165                                  170                                  175  
 Cys Tyr Asp Val Ile Ser Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys  
                                   180                                  185                                  190  
 Ala Gly Val Gly Thr Asp Leu Ile Ser Met Phe Glu Ala Ile Asn Pro  
                                   195                                  200                                  205  
 Lys Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro  
                                   210                                  215                                  220  
 Glu Ala Ser Val Phe Val Gly Gly His Phe His Lys Val Ile Gly Asn  
                                   225                                  230                                  235                                  240  
 Glu Phe Arg Asp Ile Pro Ala Met Ile Pro Ser Thr Ser Thr Leu Thr  
                                   245                                  250                                  255  
 Gly Asn His Phe Thr Ile Val Thr Leu Ser Val Cys His Phe Gly Val  
                                   260                                  265                                  270  
 Glu Leu Gly Gly Arg Phe Asn Phe  
                                   275                                  280

&lt;210&gt; 18

&lt;211&gt; 276

&lt;212&gt; PRT

&lt;213&gt; Ehrlichia chaffeensis

&lt;400&gt; 18

17

Met Asn Tyr Lys Lys Val Phe Ile Thr Ser Ala Leu Ile Ser Leu Ile  
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 Ser Ser Leu Pro Gly Val Ser Phe Ser Asp Pro Ala Gly Ser Gly Ile  
 20 25 30  
 Asn Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Ala Ser His  
 35 40 45  
 Phe Gly Val Phe Ser Ala Lys Glu Glu Arg Asn Thr Thr Val Gly Val  
 50 55 60  
 Phe Gly Leu Lys Gln Asn Trp Asp Gly Ser Ala Ile Ser Asn Ser Ser  
 65 70 75 80  
 Pro Asn Asp Val Phe Thr Val Ser Asn Tyr Ser Phe Lys Tyr Glu Asn  
 85 90 95  
 Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Met Asp Gly  
 100 105 110  
 Pro Arg Ile Glu Leu Glu Val Ser Tyr Glu Thr Phe Asp Val Lys Asn  
 115 120 125  
 Gln Gly Asn Asn Tyr Lys Asn Glu Ala His Arg Tyr Cys Ala Leu Ser  
 130 135 140  
 His Asn Ser Ala Ala Asp Met Ser Ser Ala Ser Asn Asn Phe Val Phe  
 145 150 155 160  
 Leu Lys Asn Glu Gly Leu Leu Asp Ile Ser Phe Met Leu Asn Ala Cys  
 165 170 175  
 Tyr Asp Val Val Gly Glu Gly Ile Pro Phe Ser Pro Tyr Ile Cys Ala  
 180 185 190  
 Gly Ile Gly Thr Asp Leu Val Ser Met Phe Glu Ala Thr Asn Pro Lys  
 195 200 205  
 Ile Ser Tyr Gln Gly Lys Leu Gly Leu Ser Tyr Ser Ile Ser Pro Glu  
 210 215 220  
 Ala Ser Val Phe Ile Gly Gly His Phe His Lys Val Ile Gly Asn Glu  
 225 230 235 240  
 Phe Arg Asp Ile Pro Thr Ile Ile Pro Thr Gly Ser Thr Leu Ala Gly  
 245 250 255  
 Lys Gly Asn Tyr Pro Ala Ile Val Ile Leu Asp Val Cys His Phe Gly  
 260 265 270  
 Ile Glu Met Gly  
 275

&lt;210&gt; 19

&lt;211&gt; 287

&lt;212&gt; PRT

&lt;213&gt; Ehrlichia canis

&lt;400&gt; 19

Met Lys Tyr Lys Lys Thr Phe Thr Val Thr Ala Leu Val Leu Leu Thr  
 1 5 10 15

Ser Phe Thr His Phe Ile Pro Phe Tyr Ser Pro Ala Arg Ala Ser Thr  
 20 25 30

Ile His Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Thr Ala Ser His  
 35 40 45

Phe Gly Ile Phe Ser Ala Lys Glu Glu Gln Ser Phe Thr Lys Val Leu  
 50 55 60

Val Gly Leu Asp Gln Arg Leu Ser His Asn Ile Ile Asn Asn Asn Asp  
 65 70 75 80

Thr Ala Lys Ser Leu Lys Val Gln Asn Tyr Ser Phe Lys Tyr Lys Asn  
 85 90 95

Asn Pro Phe Leu Gly Phe Ala Gly Ala Ile Gly Tyr Ser Ile Gly Asn  
 100 105 110

Ser Arg Ile Glu Leu Glu Val Ser His Glu Ile Phe Asp Thr Lys Asn  
 115 120 125

Pro Gly Asn Asn Tyr Leu Asn Asp Ser His Lys Tyr Cys Ala Leu Ser  
 130 135 140

His Gly Ser His Ile Cys Ser Asp Gly Asn Ser Gly Asp Trp Tyr Thr  
 145 150 155 160

Ala Lys Thr Asp Lys Phe Val Leu Leu Lys Asn Glu Gly Leu Leu Asp  
 165 170 175

Val Ser Phe Met Leu Asn Ala Cys Tyr Asp Ile Thr Thr Glu Lys Met  
 180 185 190

Pro Phe Ser Pro Tyr Ile Cys Ala Gly Ile Gly Thr Asp Leu Ile Ser  
 195 200 205

Met Phe Glu Thr Thr Gln Asn Lys Ile Ser Tyr Gln Gly Lys Leu Gly  
 210 215 220

Leu Asn Tyr Thr Ile Asn Ser Arg Val Ser Val Phe Ala Gly Gly His  
 225 230 235 240

Phe His Lys Val Ile Gly Asn Glu Phe Lys Gly Ile Pro Thr Leu Leu  
 245 250 255

19

Pro Asp Gly Ser Asn Ile Lys Val Gln Gln Ser Ala Thr Val Thr Leu  
 260 265 270

Asp Val Cys His Phe Gly Leu Glu Ile Gly Ser Arg Phe Phe Phe  
 275 280 285

<210> 20  
 <211> 133  
 <212> PRT  
 <213> *Ehrlichia canis*

<400> 20  
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Tyr Phe Leu Pro Asn Val Ser Tyr Ser Asn Pro Val Tyr Gly Asn Ser  
 20 25 30

Met Tyr Gly Asn Phe Tyr Ile Ser Gly Lys Tyr Met Pro Ser Val Pro  
 35 40 45

His Phe Gly Ile Phe Ser Ala Glu Glu Glu Lys Lys Lys Thr Thr Val  
 50 55 60

Val Tyr Gly Leu Lys Glu Asn Trp Ala Gly Asp Ala Ile Ser Ser Gln  
 65 70 75 80

Ser Pro Asp Asp Asn Phe Thr Ile Arg Asn Tyr Ser Phe Lys Tyr Ala  
 85 90 95

Ser Asn Lys Phe Leu Gly Phe Ala Val Ala Ile Gly Tyr Ser Ile Gly  
 100 105 110

Ser Pro Arg Ile Glu Val Glu Met Ser Tyr Glu Ala Phe Asp Val Lys  
 115 120 125

Asn Gln Gly Asn Asn  
 130

<210> 21  
 <211> 686  
 <212> DNA  
 <213> *Ehrlichia canis*

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 actataccaa atgaagacgg tattcaatct agcttttagct taatcaatca agacggtaaa 180  
 acagtaacca gccaaagattt cctagggaaa cacatgtagg ttttggttgg attctctgca 240

20

tgtaaaagca ttgccctgc agaattggga ttagtatctg aagcacttgc acaacttggt 300  
 aataatgcag acaaattaca agtaattttt attacaattg atccaaaaaa tgatactgta 360  
 gaaaaattaa aagaatttca tgaacatttt gattcaagaa ttcaaagtgt aacaggaaat 420  
 actgaagaca ttaatcaaat aattaaaaat tataaaatat atgttggaca agcagataaa 480  
 gatcatcaaa ttaaccattc tgcaataatg taccttattg acaaaaaagg atcatatctt 540  
 tcacacttca ttccagattt aaaatcacaa gaaaatcaag tagataagtt actatcttta 600  
 gttaagcagt atctgtaaat aaattcatgg aatacgttgg atgagtaggt ttttttagt 660  
 atttttagtg ctaataacat tggcat 686

<210> 22  
 <211> 618  
 <212> DNA  
 <213> Ehrlichia chaffeensis

<400> 22  
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 aatatatcaa ataaagccag cattactact agtttttctg tagtaaatca agatggaaat 180  
 acagtaaata gtcaagattt ttggggaaaa tacatgctag ttttatttgg attttcttca 240  
 tgtaaaagca tctgccctgc tgaattagga atagcatctg aagttctctc acagcttggt 300  
 aatgacacag acaagttaca agtaattttc attacaattg atccaacaaa tgatactgta 360  
 caaaaattaa aaacatttca tgaacatttt gatcctagaa ttcaaagtgt aacaggcagt 420  
 gcagaagata ttgaaaaaat aataaaaaat tacaaaaatat atgttggaca agcagataaa 480  
 gataatcaaa ttgatcactc tgccataatg tacattatcg ataaaaaagg agaatacatt 540  
 tcacactttt ctccagattt aaaatcaaca gaaaatcaag tagataagtt actatctata 600  
 ataaaacaat atctctaa 618

<210> 23  
 <211> 205  
 <212> PRT  
 <213> Ehrlichia canis

<400> 23  
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 1 5 10 15

21

Ile Phe Leu Gly Tyr Ser Tyr Ile Thr Lys Gln Gly Ile Phe Gln Thr  
                   20                  25                  30  
 Lys His His Asp Thr Pro Asn Thr Thr Ile Pro Asn Glu Asp Gly Ile  
                   35                  40                  45  
 Gln Ser Ser Phe Ser Leu Ile Asn Gln Asp Gly Lys Thr Val Thr Ser  
                   50                  55                  60  
 Gln Asp Phe Leu Gly Lys His Met Leu Val Leu Phe Gly Phe Ser Ala  
                   65                  70                  75                  80  
 Cys Lys Ser Ile Cys Pro Ala Glu Leu Gly Leu Val Ser Glu Ala Leu  
                   85                  90                  95  
 Ala Gln Leu Gly Asn Asn Ala Asp Lys Leu Gln Val Ile Phe Ile Thr  
                   100                  105                  110  
 Ile Asp Pro Lys Asn Asp Thr Val Glu Lys Leu Lys Glu Phe His Glu  
                   115                  120                  125  
 His Phe Asp Ser Arg Ile Gln Met Leu Thr Gly Asn Thr Glu Asp Ile  
                   130                  135                  140  
 Asn Gln Ile Ile Lys Asn Tyr Lys Ile Tyr Val Gly Gln Ala Asp Lys  
                   145                  150                  155                  160  
 Asp His Gln Ile Asn His Ser Ala Ile Met Tyr Leu Ile Asp Lys Lys  
                   165                  170                  175  
 Gly Ser Tyr Leu Ser His Phe Ile Pro Asp Leu Lys Ser Gln Glu Asn  
                   180                  185                  190  
 Gln Val Asp Lys Leu Leu Ser Leu Val Lys Gln Tyr Leu  
                   195                  200                  205

&lt;210&gt; 24

&lt;211&gt; 205

&lt;212&gt; PRT

&lt;213&gt; Ehrlichia chaffeensis

&lt;400&gt; 24

Met Lys Val Ile Lys Phe Ile Leu Asn Ile Cys Leu Leu Phe Ala Ala  
                   1                  5                  10                  15  
 Ile Phe Leu Gly Tyr Ser Tyr Val Thr Lys Gln Gly Ile Phe Gln Val  
                   20                  25                  30  
 Arg Asp His Asn Thr Pro Asn Thr Asn Ile Ser Asn Lys Ala Ser Ile  
                   35                  40                  45  
 Thr Thr Ser Phe Ser Leu Val Asn Gln Asp Gly Asn Thr Val Asn Ser  
                   50                  55                  60

22

Gln Asp Phe Leu Gly Lys Tyr Met Leu Val Leu Phe Gly Phe Ser Ser  
 65 70 75 80

Cys Lys Ser Ile Cys Pro Ala Glu Leu Gly Ile Ala Ser Glu Val Leu  
 85 90 95

Ser Gln Leu Gly Asn Asp Thr Asp Lys Leu Gln Val Ile Phe Ile Thr  
 100 105 110

Ile Asp Pro Thr Asn Asp Thr Val Gln Lys Leu Lys Thr Phe His Glu  
 115 120 125

His Phe Asp Pro Arg Ile Gln Met Leu Thr Gly Ser Ala Glu Asp Ile  
 130 135 140

Glu Lys Ile Ile Lys Asn Tyr Lys Ile Tyr Val Gly Gln Ala Asp Lys  
 145 150 155 160

Asp Asn Gln Ile Asp His Ser Ala Ile Met Tyr Ile Ile Asp Lys Lys  
 165 170 175

Gly Glu Tyr Ile Ser His Phe Ser Pro Asp Leu Lys Ser Thr Glu Asn  
 180 185 190

Gln Val Asp Lys Leu Leu Ser Ile Ile Lys Gln Tyr Leu  
 195 200 205

&lt;210&gt; 25

&lt;211&gt; 618

&lt;212&gt; DNA

<213> *Cowdria ruminantium*

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1) .. (615)

&lt;400&gt; 25

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 Met Lys Ala Ile Lys Phe Ile Leu Asn Leu Cys Leu Leu Phe Ala Ala  
 1 5 10 15

att ttt ttg gga tat tct tac ata aca aaa caa ggt ata ttc caa cca 96  
 Ile Phe Leu Gly Tyr Ser Tyr Ile Thr Lys Gln Gly Ile Phe Gln Pro  
 20 25 30

aaa tta cac gac tct cct gat gtt aat ata tcg aac aaa gcg gat ata 144  
 Lys Leu His Asp Ser Pro Asp Val Asn Ile Ser Asn Lys Ala Asp Ile  
 35 40 45

aat act agc ttt agc tta att aat cag gat ggt att acg ata tct agt 192  
 Asn Thr Ser Phe Ser Leu Ile Asn Gln Asp Gly Ile Thr Ile Ser Ser  
 50 55 60

aaa gac ttc ctt gga aaa cat atg tta gtc ctt ttt ggg ttt tct tct 240

23

Lys	Asp	Phe	Leu	Gly	Lys	His	Met	Leu	Val	Leu	Phe	Gly	Phe	Ser	Ser	
65					70					75					80	
tgt	aaa	act	att	tgc	ccc	atg	gaa	cta	ggg	tta	gca	ccc	aca	att	cta	288
Cys	Lys	Thr	Ile	Cys	Pro	Met	Glu	Leu	Gly	Leu	Ala	Ser	Thr	Ile	Leu	
				85					90					95		
gat	caa	ctt	ggc	aac	gaa	tct	gac	aag	tta	caa	gta	gtc	ttt	ata	act	336
Asp	Gln	Leu	Gly	Asn	Glu	Ser	Asp	Lys	Leu	Gln	Val	Val	Phe	Ile	Thr	
		100						105					110			
att	gat	cca	aca	aaa	gat	act	gta	gaa	aca	cta	aaa	gag	ttt	cac	aaa	384
Ile	Asp	Pro	Thr	Lys	Asp	Thr	Val	Glu	Thr	Leu	Lys	Glu	Phe	His	Lys	
		115					120					125				
aat	ttt	gac	tca	cgg	att	caa	atg	tta	aca	gga	aac	att	gaa	gct	att	432
Asn	Phe	Asp	Ser	Arg	Ile	Gln	Met	Leu	Thr	Gly	Asn	Ile	Glu	Ala	Ile	
	130					135					140					
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Asn	Gln	Ile	Val	Gln	Gly	Tyr	Lys	Val	Tyr	Val	Gly	Gln	Pro	Asp	Asn	
	145				150					155					160	
gat	aac	caa	att	aac	cat	tct	gga	ata	atg	tat	att	gta	gac	aag	aaa	528
Asp	Asn	Gln	Ile	Asn	His	Ser	Gly	Ile	Met	Tyr	Ile	Val	Asp	Lys	Lys	
				165					170					175		
gga	gaa	tat	tta	aca	cat	ttt	gta	cca	gat	tta	aag	tca	aaa	gag	cct	576
Gly	Glu	Tyr	Leu	Thr	His	Phe	Val	Pro	Asp	Leu	Lys	Ser	Lys	Glu	Pro	
			180					185					190			
caa	gtg	gat	aaa	tta	ctt	tct	tta	att	aag	cag	tat	ctt	taa			618
Gln	Val	Asp	Lys	Leu	Leu	Ser	Leu	Ile	Lys	Gln	Tyr	Leu				
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&lt;210&gt; 26

&lt;211&gt; 205

&lt;212&gt; PRT

&lt;213&gt; Cowdria ruminantium

&lt;400&gt; 26

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Ile	Phe	Leu	Gly	Tyr	Ser	Tyr	Ile	Thr	Lys	Gln	Gly	Ile	Phe	Gln	Pro
		20						25					30		

Lys	Leu	His	Asp	Ser	Pro	Asp	Val	Asn	Ile	Ser	Asn	Lys	Ala	Asp	Ile
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Asn	Thr	Ser	Phe	Ser	Leu	Ile	Asn	Gln	Asp	Gly	Ile	Thr	Ile	Ser	Ser
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24

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 Cys Lys Thr Ile Cys Pro Met Glu Leu Gly Leu Ala Ser Thr Ile Leu  
 85 90 95  
 Asp Gln Leu Gly Asn Glu Ser Asp Lys Leu Gln Val Val Phe Ile Thr  
 100 105 110  
 Ile Asp Pro Thr Lys Asp Thr Val Glu Thr Leu Lys Glu Phe His Lys  
 115 120 125  
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&lt;210&gt; 27

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&lt;212&gt; DNA

<213> *Cowdria ruminantium*

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&lt;400&gt; 27

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 Asn Ala Ala Ile Ala Ser Thr Asp Ser Ser Glu Asp Lys Gln Tyr Ile  
 20 25 30  
 tta att ggt act ggt tct atg act gga gta tat tat cct ata gga ggt 144  
 Leu Ile Gly Thr Gly Ser Met Thr Gly Val Tyr Tyr Pro Ile Gly Gly  
 35 40 45  
 agc ata tgt agg ttt att gca tct gat tat ggt aat gat aat aac agc 192  
 Ser Ile Cys Arg Phe Ile Ala Ser Asp Tyr Gly Asn Asp Asn Asn Ser  
 50 55 60

25

ata gtt tgt tct ata tct tct aca act ggt agc gta tat aat ctt aat	240
Ile Val Cys Ser Ile Ser Ser Thr Thr Gly Ser Val Tyr Asn Leu Asn	
65 70 75 80	
tct atg cgt tat gca aat atg gat ata ggt att att caa tct gat tta	288
Ser Met Arg Tyr Ala Asn Met Asp Ile Gly Ile Ile Gln Ser Asp Leu	
85 90 95	
gag tac tat gca tat aat ggt att ggt tta tat gaa aaa atg cca gca	336
Glu Tyr Tyr Ala Tyr Asn Gly Ile Gly Leu Tyr Glu Lys Met Pro Ala	
100 105 110	
atg agg cat cta aga ata tta tct tca tta cat aaa gaa tat ctt aca	384
Met Arg His Leu Arg Ile Leu Ser Ser Leu His Lys Glu Tyr Leu Thr	
115 120 125	
att gtt gtt agg gcg aat tct aat ata tca gtt att gat gat ata aaa	432
Ile Val Val Arg Ala Asn Ser Asn Ile Ser Val Ile Asp Asp Ile Lys	
130 135 140	
ggc aaa aga gtt aat att ggt agt cct ggt act ggt gta aga ata gca	480
Gly Lys Arg Val Asn Ile Gly Ser Pro Gly Thr Gly Val Arg Ile Ala	
145 150 155 160	
atg tta aaa ttg tta aat gaa aaa gga tgg gga aga aaa gat ttt gct	528
Met Leu Lys Leu Leu Asn Glu Lys Gly Trp Gly Arg Lys Asp Phe Ala	
165 170 175	
gtt atg gca gaa tta aaa tca tca gag caa gct caa gca tta tgt gat	576
Val Met Ala Glu Leu Lys Ser Ser Glu Gln Ala Gln Ala Leu Cys Asp	
180 185 190	
aat aaa att gat gtg atg gta gat gtt gtt gga cat cct aat gct gca	624
Asn Lys Ile Asp Val Met Val Asp Val Val Gly His Pro Asn Ala Ala	
195 200 205	
att caa gaa gca gca gca act tgt gat ata aaa ttt att tct tta gat	672
Ile Gln Glu Ala Ala Ala Thr Cys Asp Ile Lys Phe Ile Ser Leu Asp	
210 215 220	
gat gat ctc ata gat aaa tta cat act aag tat ccc tat tat aaa agg	720
Asp Asp Leu Ile Asp Lys Leu His Thr Lys Tyr Pro Tyr Tyr Lys Arg	
225 230 235 240	
gat att att agt ggt gcg tta tac agt aac tta cct gat ata caa act	768
Asp Ile Ile Ser Gly Ala Leu Tyr Ser Asn Leu Pro Asp Ile Gln Thr	
245 250 255	
gtt tca gta aaa gct tct tta ata aca act act gaa tta agc aat gag	816
Val Ser Val Lys Ala Ser Leu Ile Thr Thr Thr Glu Leu Ser Asn Glu	
260 265 270	
ttg gcc tat aaa gtt gtt aaa tct ttg gtt agc cat tta cat gaa cta	864
Leu Ala Tyr Lys Val Val Lys Ser Leu Val Ser His Leu His Glu Leu	
275 280 285	

26

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cag tca gat att aca cct tta cat gac ggt gca aaa cgt tat tat aag 960  
 Gln Ser Asp Ile Thr Pro Leu His Asp Gly Ala Lys Arg Tyr Tyr Lys  
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gaa att gga gtt ata aaa taa 981  
 Glu Ile Gly Val Ile Lys  
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&lt;210&gt; 28

&lt;211&gt; 326

&lt;212&gt; PRT

&lt;213&gt; Cowdria ruminantium

&lt;400&gt; 28

Met Lys Lys Ile Leu Val Thr Phe Leu Val Val Val Asn Val Phe Cys  
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Asn Ala Ala Ile Ala Ser Thr Asp Ser Ser Glu Asp Lys Gln Tyr Ile  
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Leu Ile Gly Thr Gly Ser Met Thr Gly Val Tyr Tyr Pro Ile Gly Gly  
 35 40 45

Ser Ile Cys Arg Phe Ile Ala Ser Asp Tyr Gly Asn Asp Asn Asn Ser  
 50 55 60

Ile Val Cys Ser Ile Ser Ser Thr Thr Gly Ser Val Tyr Asn Leu Asn  
 65 70 75 80

Ser Met Arg Tyr Ala Asn Met Asp Ile Gly Ile Ile Gln Ser Asp Leu  
 85 90 95

Glu Tyr Tyr Ala Tyr Asn Gly Ile Gly Leu Tyr Glu Lys Met Pro Ala  
 100 105 110

Met Arg His Leu Arg Ile Leu Ser Ser Leu His Lys Glu Tyr Leu Thr  
 115 120 125

Ile Val Val Arg Ala Asn Ser Asn Ile Ser Val Ile Asp Asp Ile Lys  
 130 135 140

Gly Lys Arg Val Asn Ile Gly Ser Pro Gly Thr Gly Val Arg Ile Ala  
 145 150 155 160

Met Leu Lys Leu Leu Asn Glu Lys Gly Trp Gly Arg Lys Asp Phe Ala  
 165 170 175

Val Met Ala Glu Leu Lys Ser Ser Glu Gln Ala Gln Ala Leu Cys Asp  
 180 185 190

27

Asn Lys Ile Asp Val Met Val Asp Val Val Gly His Pro Asn Ala Ala  
 195 200 205  
 Ile Gln Glu Ala Ala Ala Thr Cys Asp Ile Lys Phe Ile Ser Leu Asp  
 210 215 220  
 Asp Asp Leu Ile Asp Lys Leu His Thr Lys Tyr Pro Tyr Tyr Lys Arg  
 225 230 235 240  
 Asp Ile Ile Ser Gly Ala Leu Tyr Ser Asn Leu Pro Asp Ile Gln Thr  
 245 250 255  
 Val Ser Val Lys Ala Ser Leu Ile Thr Thr Thr Glu Leu Ser Asn Glu  
 260 265 270  
 Leu Ala Tyr Lys Val Val Lys Ser Leu Val Ser His Leu His Glu Leu  
 275 280 285  
 His Gly Ile Thr Gly Ala Leu Arg Asn Leu Thr Val Lys Asp Met Val  
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 1 5 10 15  
 gca ttt gtt gca cct act gct gta att ata ggt gat gtt tgt gta aat 96  
 Ala Phe Val Ala Pro Thr Ala Val Ile Ile Gly Asp Val Cys Val Asn  
 20 25 30  
 gac aag tgt agc att tgg tat aac tca gta tta cgt gga gat gta ggc 144  
 Asp Lys Cys Ser Ile Trp Tyr Asn Ser Val Leu Arg Gly Asp Val Gly  
 35 40 45  
 caa att gtt att ggt gta ggt act aat att caa gat ggg aca ata ata 192  
 Gln Ile Val Ile Gly Val Gly Thr Asn Ile Gln Asp Gly Thr Ile Ile  
 50 55 60

28

cat gtt gat agg aaa tat ggt aat acg aat att ggc aaa aag gtt act 240  
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 65 70 75 80

att ggg cat ggg tgt ata tta cat gct tgt gag ata caa gat tat gtg 288  
 Ile Gly His Gly Cys Ile Leu His Ala Cys Glu Ile Gln Asp Tyr Val  
 85 90 95

ctt gtt gga atg gga tct att att atg gat aac gtt gtg gtt gaa aag 336  
 Leu Val Gly Met Gly Ser Ile Ile Met Asp Asn Val Val Val Glu Lys  
 100 105 110

aat gca atg gtg gct gct gga tca tta gtg gta aga ggt aaa gtt gtg 384  
 Asn Ala Met Val Ala Ala Gly Ser Leu Val Val Arg Gly Lys Val Val  
 115 120 125

aaa act ggt gaa tta tgg gct ggt agg cct gca caa ttt tta aga atg 432  
 Lys Thr Gly Glu Leu Trp Ala Gly Arg Pro Ala Gln Phe Leu Arg Met  
 130 135 140

ttg tct agt gat gaa att aaa gag ata agt aaa tct gct gat aac tat 480  
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 145 150 155 160

ata gag ctt gcc agt gat tac ata act ggt aag ttg taa 519  
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 165 170

&lt;210&gt; 30

&lt;211&gt; 172

&lt;212&gt; PRT

&lt;213&gt; Cowdria ruminantium

&lt;400&gt; 30

Met Asn Ile Phe Asn Tyr Met Gln Ile Met Pro Asn Ile Ser Val Asp  
 1 5 10 15

Ala Phe Val Ala Pro Thr Ala Val Ile Ile Gly Asp Val Cys Val Asn  
 20 25 30

Asp Lys Cys Ser Ile Trp Tyr Asn Ser Val Leu Arg Gly Asp Val Gly  
 35 40 45

Gln Ile Val Ile Gly Val Gly Thr Asn Ile Gln Asp Gly Thr Ile Ile  
 50 55 60

His Val Asp Arg Lys Tyr Gly Asn Thr Asn Ile Gly Lys Lys Val Thr  
 65 70 75 80

Ile Gly His Gly Cys Ile Leu His Ala Cys Glu Ile Gln Asp Tyr Val  
 85 90 95

Leu Val Gly Met Gly Ser Ile Ile Met Asp Asn Val Val Val Glu Lys  
 100 105 110

29

Asn Ala Met Val Ala Ala Gly Ser Leu Val Val Arg Gly Lys Val Val  
 115 120 125

Lys Thr Gly Glu Leu Trp Ala Gly Arg Pro Ala Gln Phe Leu Arg Met  
 130 135 140

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Ile Glu Leu Ala Ser Asp Tyr Ile Thr Gly Lys Leu  
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 20 25 30

act aca ttg gat aag gat gca gtt ata tct ata gtt gag gaa tat ata 144  
 Thr Thr Leu Asp Lys Asp Ala Val Ile Ser Ile Val Glu Glu Tyr Ile  
 35 40 45

acc aat tat cct cag agg gta ata gat tta ctt act aca ggc caa gca 192  
 Thr Asn Tyr Pro Gln Arg Val Ile Asp Leu Leu Thr Thr Gly Gln Ala  
 50 55 60

caa gca gaa aga gca gag ctt act gaa aat att aaa aaa tat aaa tct 240  
 Gln Ala Glu Arg Ala Glu Leu Thr Glu Asn Ile Lys Lys Tyr Lys Ser  
 65 70 75 80

gag ctt gaa gat att gca tac cca tct gct ggc aat aaa gac agt aaa 288  
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 85 90 95

att gca ttt att gag ttc ttc gat tac tct tgt ggt tat tgt aaa atg 336  
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 100 105 110

atg ttt gaa gat atc aaa caa att ata aaa gat ggt aag gta cgt gtt 384  
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 115 120 125

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att ttt aga gat ttt cca ata ctt ggg gaa tct tct tta aag gct gtt 432  
 Ile Phe Arg Asp Phe Pro Ile Leu Gly Glu Ser Ser Leu Lys Ala Val  
 130 135 140

aaa gca gca ttg gct gta cat ctt atc aat cca agt aaa tac ttg gac 480  
 Lys Ala Ala Leu Ala Val His Leu Ile Asn Pro Ser Lys Tyr Leu Asp  
 145 150 155 160

ttc tat tat gca gca tta aat cat aaa cag cca ttt aat gat gaa tct 528  
 Phe Tyr Tyr Ala Ala Leu Asn His Lys Gln Pro Phe Asn Asp Glu Ser  
 165 170 175

ata ctt aat ata gtt aaa tca ctt gaa att tca gaa gag gaa ttt aaa 576  
 Ile Leu Asn Ile Val Lys Ser Leu Glu Ile Ser Glu Glu Phe Lys  
 180 185 190

gat tct tta tct aaa aat tct agt act att gat aag atg ata gag tcc 624  
 Asp Ser Leu Ser Lys Asn Ser Ser Thr Ile Asp Lys Met Ile Glu Ser  
 195 200 205

act aga aat ctg gct gag aag tta aat atc aga ggt act cct gct ctt 672  
 Thr Arg Asn Leu Ala Glu Lys Leu Asn Ile Arg Gly Thr Pro Ala Leu  
 210 215 220

ata ata ggt gat gca ttc att ggg gga gct gca gat tta tca act tta 720  
 Ile Ile Gly Asp Ala Phe Ile Gly Gly Ala Ala Asp Leu Ser Thr Leu  
 225 230 235 240

aga agt aaa ata gta gaa cag cag gaa caa taa 753  
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 245 250

&lt;210&gt; 32

&lt;211&gt; 250

&lt;212&gt; PRT

&lt;213&gt; Cowdria ruminantium

&lt;400&gt; 32

Met Met Ile Arg Ile Phe Leu Leu Leu Gly Leu Val Leu Leu Val Ala  
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Ser Phe Pro Leu Leu Asn Asn Trp Leu Ser Asn His Ser Gly Lys Ser  
 20 25 30

Thr Thr Leu Asp Lys Asp Ala Val Ile Ser Ile Val Glu Glu Tyr Ile  
 35 40 45

Thr Asn Tyr Pro Gln Arg Val Ile Asp Leu Leu Thr Thr Gly Gln Ala  
 50 55 60

Gln Ala Glu Arg Ala Glu Leu Thr Glu Asn Ile Lys Lys Tyr Lys Ser  
 65 70 75 80

31

Glu Leu Glu Asp Ile Ala Tyr Pro Ser Ala Gly Asn Lys Asp Ser Lys  
85 90 95

Ile Ala Phe Ile Glu Phe Phe Asp Tyr Ser Cys Gly Tyr Cys Lys Met  
100 105 110

Met Phe Glu Asp Ile Lys Gln Ile Ile Lys Asp Gly Lys Val Arg Val  
115 120 125

Ile Phe Arg Asp Phe Pro Ile Leu Gly Glu Ser Ser Leu Lys Ala Val  
130 135 140

Lys Ala Ala Leu Ala Val His Leu Ile Asn Pro Ser Lys Tyr Leu Asp  
145 150 155 160

Phe Tyr Tyr Ala Ala Leu Asn His Lys Gln Pro Phe Asn Asp Glu Ser  
165 170 175

Ile Leu Asn Ile Val Lys Ser Leu Glu Ile Ser Glu Glu Glu Phe Lys  
180 185 190

Asp Ser Leu Ser Lys Asn Ser Ser Thr Ile Asp Lys Met Ile Glu Ser  
195 200 205

Thr Arg Asn Leu Ala Glu Lys Leu Asn Ile Arg Gly Thr Pro Ala Leu  
210 215 220

Ile Ile Gly Asp Ala Phe Ile Gly Gly Ala Ala Asp Leu Ser Thr Leu  
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Arg Ser Lys Ile Val Glu Gln Gln Glu Gln  
245 250

&lt;210&gt; 33

&lt;211&gt; 450

&lt;212&gt; DNA

&lt;213&gt; Cowdria ruminantium

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(447)

&lt;400&gt; 33

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Met His Arg Ser Asn Ile Ile Glu Ile Phe Ile Gly Phe Leu Val Leu  
1 5 10 15

gca gga gca ata tct att ggg ata ata gca ttt aac aaa tta cca tat 96  
Ala Gly Ala Ile Ser Ile Gly Ile Ile Ala Phe Asn Lys Leu Pro Tyr  
20 25 30

aaa aat acc ttg cgt aat tgt tat aca gtt aaa gca ttt ttc tca aat 144  
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BNSDOCID: <WO\_\_\_0065063A3\_IA>

33

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115 120 125

His Thr His Ser Asp Leu Ser Phe Asn Ala Ile Ile Ala Lys Ile Ile  
130 135 140

Asp Ser Leu Ile Lys  
145

## INTERNATIONAL SEARCH REPORT

Intern al Application No

PCT/US 00/10886

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/31 C07K14/29 A61K39/02 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 16554 A (UNIV FLORIDA) 23 April 1998 (1998-04-23) the whole document	1-24
X	--- BOWIE MICHAEL V ET AL: "Potential value of major antigenic protein 2 for serological diagnosis of heartwater and related Ehrlichial infections." CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, vol. 6, no. 2, March 1999 (1999-03), pages 209-215, XP000939015 ISSN: 1071-412X the whole document --- -/-	1-4, 7-13, 21-24

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

5 September 2000

Date of mailing of the international search report

22. 12. 2000

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ANDRES S.M.

## INTERNATIONAL SEARCH REPORT

Internat'l Application No

PCT/US 00/10886

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NYIKA A ET AL.: "A DNA vaccine protects mice against the rickettsial agent Cowdria ruminantium." PARASITE IMMUNOLOGY (OXFORD), vol. 20, no. 3, March 1998 (1998-03), pages 111-119, XP000939081 ISSN: 0141-9838 the whole document ----	1-4, 6-14, 17-19
X	MAHAN S M ET AL: "Molecular cloning of a gene encoding the immunogenic 21 kDa protein of Cowdria ruminantium." MICROBIOLOGY (READING), vol. 140, no. 8, 1994, pages 2135-2142, XP000939016 the whole document -----	1-4, 7-13, 21-24

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/10886

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-24

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 10 to 19 are directed to a method of treatment of the human/animal body, and claim 20 (as far as an in vivo method is concerned) is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-24

1.1. Claims: 1-2,6-7,10-11,17-19,21-22 (all partially)

A composition comprising a polynucleotide encoding an antigen from *Rickettsia* spp. and methods for using it in protection of a host against a disease or death, or in diagnostic.

1.2. Claims: 1-4,6-13,17-24 (all partially), and claims 5, 15 (totally)

Compositions comprising SEQ IDs 3,4; 7,14; 8,15; 9,16; 10,17; 11,18 and 22,24 (corresponding to the MAP1, VSA1 to VSA5 and MAP2 antigens from *Ehrlichia chaffeensis*) and methods for using them in protection of a host against a disease or death, or in diagnostic.

1.3. Claims: 1-4,6-13,17-24 (all partially)

Compositions comprising SEQ IDs 12,19; 13,20 and 21,23 (corresponding to the VSA1, VSA2 and MAP2 antigens from *Ehrlichia canis*) and methods for using them in protection of a host against a disease or death, or in diagnostic.

1.4. Claims: 1-4,6-13,17-19, 21-24 (all partially) and claim 16 (totally)

A compositions comprising SEQ IDs 4 and 5 (corresponding to the MSP-4 antigen from *Anaplasma marginale*) and methods for using it in protection of a host against a disease or death, or in diagnostic.

1.5. Claims: 1-4,6-13,17-19, 21-24 (all partially) and claim 14 (totally)

Compositions comprising SEQ IDs 1,2 and 25,26 (corresponding to the antigens MAP1 and MAP2 from *Cowdria ruminantium*) and methods for using them in protection of a host against a disease or death, or in diagnostic.

2. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 27 and 28 (corresponding to the lhwhorf3 antigen from *Cowdria ruminantium*) and methods

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

for using it in protection of a host against a disease or death, or in diagnostic.

3. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 29 and 30 (corresponding to the 4hworfl antigen from Cowdria ruminantium) and methods for using it in protection of a host against a disease or death, or in diagnostic.

4. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 31 and 32 (corresponding to the 18hworfl antigen from Cowdria ruminantium) and methods for using it in protection of a host against a disease or death, or in diagnostic.

5. Claims: 1-4,6-13,17-19,21-24 (all partially)

A composition comprising SEQ IDs 33 and 34 (corresponding to the 3gdorf3 antigen from Cowdria ruminantium) and methods for using it in protection of a host against a disease or death, or in diagnostic.

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched without effort justifying an additional fee.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/10886

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9816554 A	23-04-1998	US 6025338 A	15-02-2000
		AU 4913097 A	11-05-1998
		ZA 9709320 A	16-03-1999
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Form PCT/ISA/210 (patent family annex) (July 1992)

